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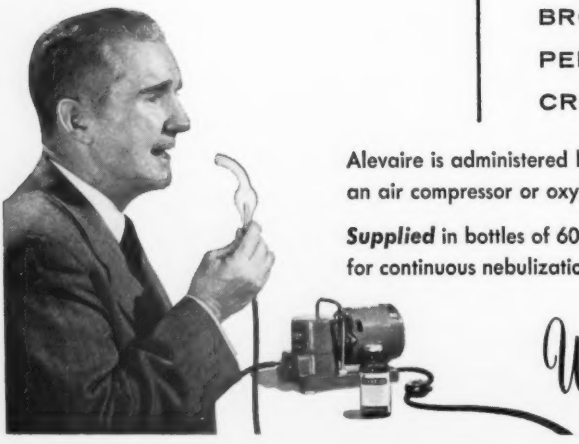

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THE YALE JOURNAL
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EXPERIMENTAL PYELONEPHRITIS. III. THE INFLUENCE OF LOCALIZED INJURY IN DIFFERENT PARTS OF THE KIDNEY ON SUSCEPTIBILITY TO BACILLARY INFECTION¶

Most cases of pyelonephritis in man are caused by the coliform group of bacilli. This infection cannot be produced in normal animals even when large numbers of the bacteria are inoculated into the blood stream or into the urinary passages. Pathogenic staphylococci, on the other hand, when inoculated by the intravenous route, are capable of infecting the normal kidney, causing multiple abscesses in all zones of the kidneys.[4,6] The pathological lesion of staphylococcal pyelonephritis is characterized early by abscess formation and late by the presence of large scars and gross deformities.

In 1956 deNavasquez made the significant observation that rabbits which had recovered from staphylococcal infection of the kidneys had now become susceptible to hematogenous pyelonephritis due to *E. coli* or *Pseudomonas pyocyanea*.[5] His studies showed that the bacillary pyogenic infections tended to develop in areas previously damaged by staphylococci, in and around tubules partially obstructed and distended by the scars. The work reported here was undertaken in an effort to learn more about the way in which localized injury of kidney parenchyma may affect its susceptibility to coliform bacterial infection. Instead of a preceding staphylococcal infection, lesions were placed selectively by means of an electric cautery. A preliminary note has appeared elsewhere.[8]

* Fellow of the Kellogg Foundation. This work is part of a thesis presented by Dr. Rocha at the University of Bahia.

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MATERIALS AND METHODS

Except where specified, the materials and methods were the same as described in preceding articles in this series.[7,8]

Production of the renal scar. The cautery tip was a fine insulated wire attached to one of the electrodes of a Davis-Bovie Electro Surgical Unit (The Liebell-Flarsheim Co.). Approximately 2 mm. at the tip of this wire was uncovered. The instrument was set at 25 volts, and the time allowed for heat coagulation to occur was 1-2 seconds.

Procedure: Animals were anesthetized by intravenous administration of 30-50 mg. of pentobarbital sodium in sterile saline. Supplementary ether inhalation anesthesia was employed when needed for adequate relaxation. For technical reasons the left kidney was the one selected for injury in all experiments. An electrode was placed beneath this side of the animal, in the lumbar area. With an electric clipper the hair of the abdomen was removed, and the operative site cleansed with 70 per cent alcohol. After gentle palpation of the kidney, a 5-cm. incision was made over it, and the organ delivered into the operative wound. Cortical injury was produced by inserting the cautery only to the depth of the uninsulated portion of the cautery. To injure the papilla the cautery wire was inserted into the anterior surface of the kidney 1.3 cm. medial to the outer margin, midway between the upper and lower poles, to a depth of 1.3 cm. Injury of the medulla, but not in the papilla, was accomplished by inserting the cautery as just described, but 0.5 cm. below the midzone.

After producing one or more areas of injury, the wound was closed, using silk sutures for the peritoneum and muscles, and metal clips for the skin. Merthiolate solution (1/1000) was applied over the surgical wound.

Removal of kidney for bacteriological and pathological studies. Under pentobarbital anesthesia, the abdominal wall was cleansed with 70 per cent alcohol. Using sterile instruments, the abdominal wall was opened by a large incision and kidneys were well visualized. After collecting urine and blood in some cases, kidneys were removed and put in sterile Petri dishes for close macroscopic examination and sectioning. With sterile gauze and a sterile (Virchow) brain knife, a midline longitudinal section was made. Half of the kidney was placed in a Ten Broeck grinder or a tube of a "Precision" homogenizer, and ground in 9 ml. of nutrient broth (Difco) until a smooth suspension was obtained. Serial ten-fold dilutions in nutrient broth were then carried out, and pour plates were made according to the expected number of organisms. Plates were incubated for at least 48 hours and the numbers of colonies were recorded. Confirmation of the presence of *E. coli* was made by sub-culturing some colonies on desoxycholate agar, Kligler's iron agar, and Simmons' citrate agar (Difco). The remaining half of the kidney was fixed in 10 per cent formol for microscopic sections.

Urine culture. Immediately after opening the abdominal cavity a small quantity of urine was aspirated through the bladder wall, under aseptic conditions. One loopful of this was streaked on a blood agar plate and another on desoxycholate agar. In some cases, serial dilution of the urine was carried out in nutrient broth, and pour plates were made for the final bacteriological count. Organisms present in the plates were identified as already described.

Blood culture. In some cases, blood cultures were taken by direct heart puncture before any manipulation of the kidney or bladder of the animal. Blood was also obtained at times from a marginal ear vein which had had no previous bacterial injection. Blood

agar pour plates were prepared, containing 1 ml. of blood. These were incubated for 48 hours, before counting the colonies of bacteria. Identification of any bacterial growth was by the usual methods.

EXPERIMENTAL

Relation of site of injury to susceptibility to infection. (a) *Cortex.* By inserting the cautery wire to a depth of about 2 mm. it was possible to produce an area of coagulation necrosis 2-4 mm. in diameter in cortical

TABLE 1. CORTICAL LESIONS

Rabbit No.	*No. of lesions*	*Time between injury and injection of bacteria*	*Colonies E. coli*		
			Lt. kidney	*Rt. kidney*	*Bladder urine (per ml.)*
1	10	1 week	0	0	0
2	20	"	0	0	0
3	20	"	0	0	0
4	20	"	0	0	0
5	1	2 weeks	10^5	0	10^4
6	1	"	0	0	0
7	10	"	0	0	0
8	20	"	0	0	0
9	20	"	0	0	0
10	20	"	0	0	0
11	20	6 weeks	0	0	0
12	20	"	0	0	0
13	20	"	0	0	0

Results of bacteriological examination of kidneys and bladder urine one week after intravenous injection of *E. coli* in rabbits with cautery lesions of the cortex. (The lesion in rabbit No. 5 was found later to be located in the upper portion of the medulla and was not confined to cortical tissue.)

tissue. This was done in several experiments involving, in all, 13 rabbits. In most of them 10 to 20 lesions were produced in the same kidney. The appearance of such cortical lesions is shown in Figures 1 and 2. After an interval of 1 to 6 weeks the bacterial inoculation was then given intravenously. One week later both kidneys were removed and examined for the presence of infection. The results are given in Table 1. It will be seen that none of the normal kidneys became infected, and only 1 of 13 (No. 5) of those with cortical scars did. In this one animal, it was later evident upon microscopic examination of the tissue sections that the lesion had erroneously been placed in the medulla.

It seems possible to conclude, then, that multiple injuries of cortical tissue do not affect the susceptibility of the kidney to this kind of infection.

(b) *Medulla.* In an initial series of tests, single medullary lesions were produced in 23 animals, 6 in the papilla, the remainder toward the lower

TABLE 2. MEDULLARY LESIONS

Rabbit No.	*Time between injury and injection of bacteria*	*Colonies E. coli*		
		Lt. kidney	*Rt. kidney*	*Bladder urine (per ml.)*
1	1 week	$>10^7$	$>10^5$	—
2	"	10^4	0	+
3	"	10^5	10^5	+
4	"	10^2	0	0
5	"	10^3	0	0
6	"	10^5	0	+
7	"	10^4	0	+
8	"	10^7	0	—
9	"	0	0	—
10	"	10^5	0	10^7
11	"	10^7	0	contam.
12	"	10^7	0	10^6
13	"	10^5	0	10^4
14	"	10^7	0	10^6
15	"	10^6	0	10^5
16	"	$>10^7$	0	$>10^7$
17	"	$>10^7$	0	—
18	2 weeks	$>10^7$	$>10^6$	—
19	"	0	0	—
20	"	10^6	10^2	—
21	"	$>10^8$	0	10^6
22	"	$>10^8$	0	10^5
23	"	0	0	0

Results of bacteriological examination of kidneys and bladder urine one week after the intravenous injection of *E. coli* in rabbits with cautery injuries of the medulla. + denotes positive culture but quantitative estimate not made.

pole of the kidney. After intervals of 7 to 14 days, injections of bacterial culture were given, and 7 days later both kidneys were examined for the occurrence of infection. The results are given in Table 2, where it is shown that significant numbers of bacteria, i.e., 10^4 or more, were found in 18 of 23 animals. Infection of the contralateral kidney had also occurred in 3 of the 18.

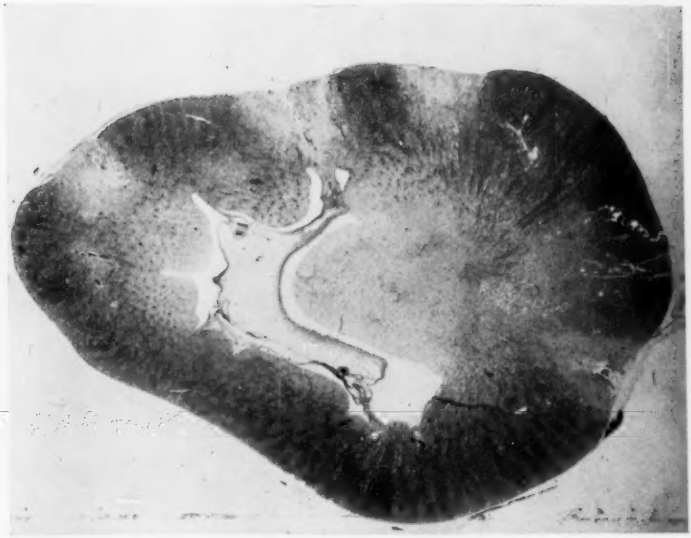

FIG. 1. The appearance of four separate areas of injury to the cortex. The lesions are 14 days old. There is disorganization of cortical structure with the early formation of surface depressions.

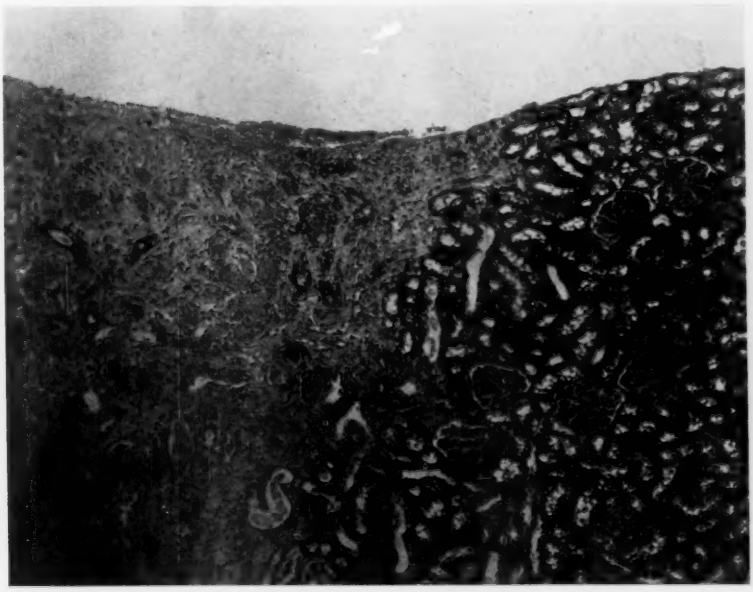

FIG. 2. A magnification of one of the lesions seen in Figure 1 showing the border between injured (left) and normal cortex. In the area of injury there is minimal lymphocytic infiltration. Necrotic tubules are still recognizable structural units.

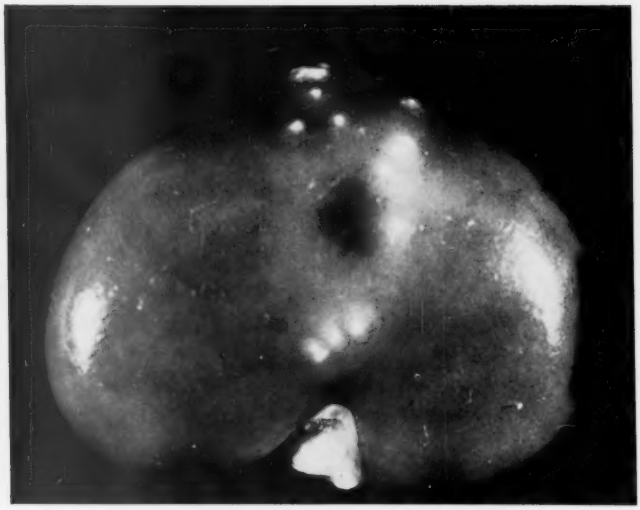

FIG. 3. Distribution of infection when cautery injury is placed in the center of the renal papilla. Note collection of abscesses across the mid-zone of the cortex one week after the introduction of *E. coli* intravenously. The infected portion of kidney bulges above the level of normal kidney.

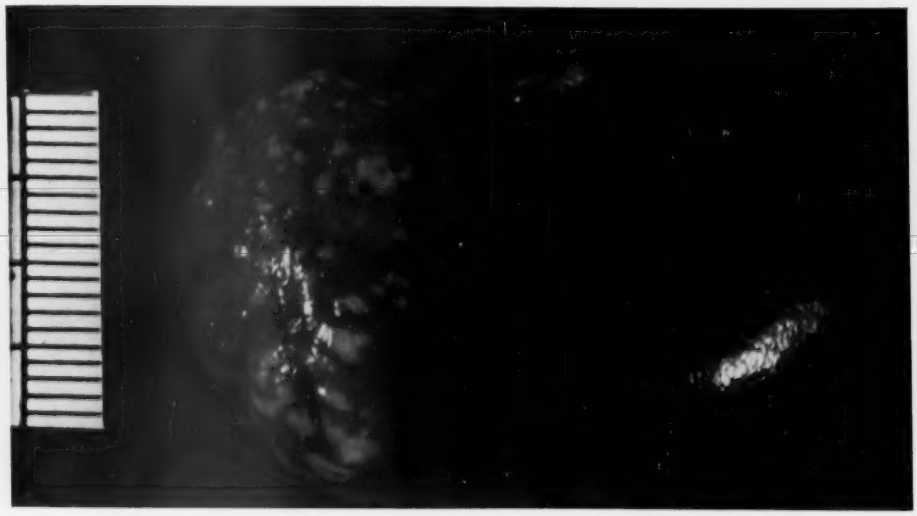

FIG. 4. Distribution of infection when cautery injury had been placed in the medulla toward one pole of the kidney. Note collection of abscesses over that entire portion at one week.

FIG. 5. Cut section illustrating distribution of a 7-day infection. Site of cautery injury appears as a roughly circular necrotic area in the medulla toward the left. Radiating out from this through the outer medulla are alternating light and dark streaks. The corresponding portion of the cortex is pale and swollen, due to necrosis and abscess formation.

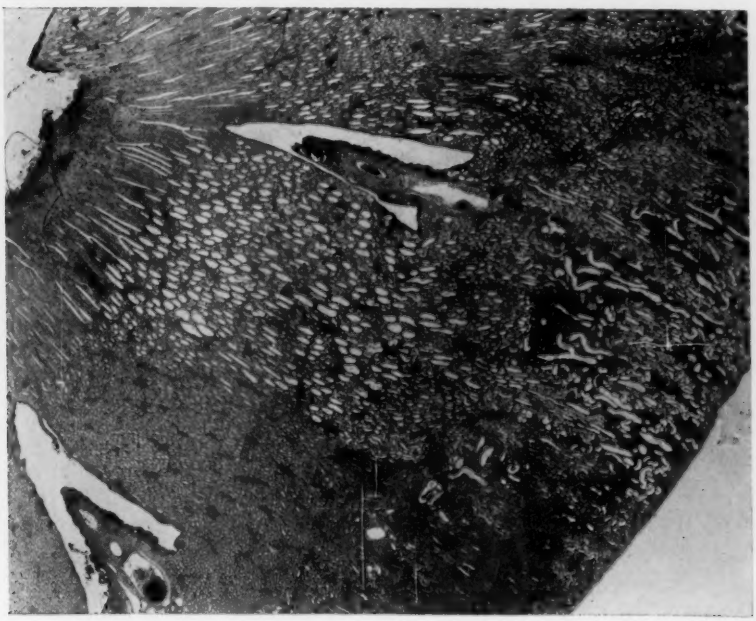

FIG. 6. The "intrarenal hydronephrosis" caused by cautery injury in the medulla. Actual site of injury is at the defect in tip of papilla, upper left. This low power magnification shows the abrupt transition between affected and nonaffected areas of renal substance 7 days after the injury.

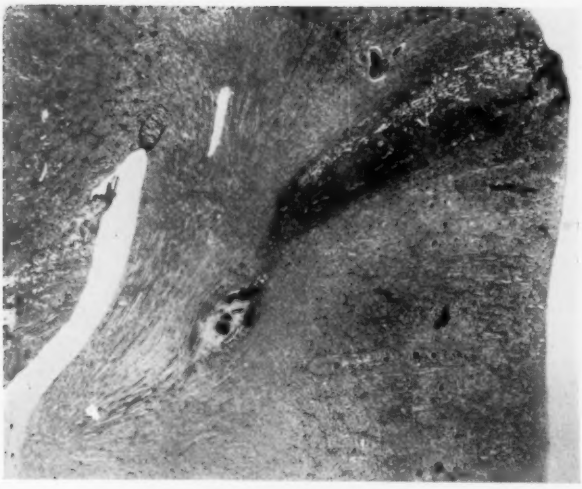

FIG. 7. Microscopic view showing cautery injury at base of papilla and, radiating out from it, a wedge-shaped area of dilated tubules in which there are many confluent abscesses.

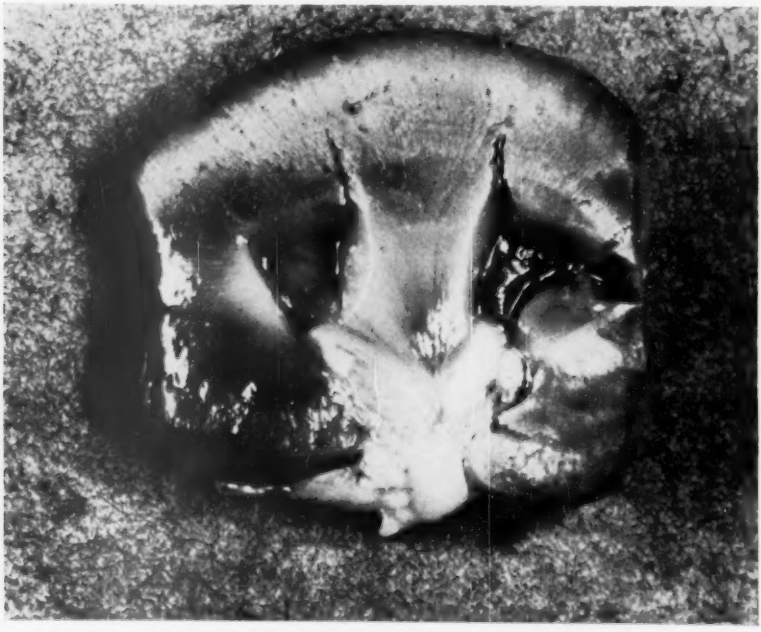

FIG. 8. Kidney with pieces removed to determine the site of earliest bacterial growth. There is a small amount of hemorrhage visible in the medulla at the right indicating the site of previous injury.

In the course of subsequent experiments, designed to study other problems, additional animals were subjected to medullary injury followed by inoculation of bacterial culture as outlined in the preceding paragraph. The total experience included 65 rabbits. Of 26 with single lesions in the papilla, 21 became infected; of 39 with lesions in the medulla near one pole, 35 became infected. Thus about 85 per cent of kidneys with small cautery injuries in medullary tissue were found to be susceptible to hematogenous infection with *E. coli*. In only one instance in this latter group was the contralateral kidney also found to be infected, but in most of these experiments the kidneys were examined 24 to 72 hours after the intravenous inoculation, and this short time interval may have limited the opportunity for infection to develop in the normal kidney. This subject will be considered in the Discussion.

Gross appearance of infection associated with injury of the medulla. The presence of infection could usually be ascertained by inspection of the external surface of the kidney as early as 36-48 hours after injection of the bacteria. By that time clusters of small yellow, slightly raised areas of purulent exudate were visible beneath the capsule. These usually were 1-3 mm. in diameter and occasionally were coalescent. The distribution of the abscesses was in relation to the location of the cautery injury. In the case of centrally placed lesions they tended to fan outward to the lateral margin as shown in Figure 3; whereas with lesions located in the medulla near the lower pole they were located over that part of the kidney as shown in Figure 4. The area of gross infection was always sharply defined, and there was little or no tendency for it to spread into neighboring areas. Examination of a cut section of the kidney during this acute phase of the infection usually showed a small yellow-white necrotic area in the medulla or papilla, the site of the cautery injury. Radiating out from this toward the surface of the kidney was a wedge of infection corresponding in distribution to the nephrons whose collecting system had been injured by the cautery. The infected area was swollen, as evidenced by some bulging beneath the capsule and a tendency for its cut surface to stand above the level of adjacent tissue. The characteristic appearance was alternating yellow and red streaks in the medulla, radiating out to the inner cortical zone; beyond this in the outer cortex there were collections of yellow material—the abscesses visible on the surface of the kidney. This is illustrated in Figure 5. Spread of the infection to perirenal structures was never observed.

Microscopic appearance of the lesion. The local area of injury caused by the cautery, as shown in Figures 1 and 2, consists of simple coagulation necrosis. Medullary injury, however, has a secondary effect which may be of prime importance in connection with its enhancement of susceptibility to

infection: obstruction of the tubules proximal to it. This is illustrated in Figure 6, where the zone of "intrarenal hydronephrosis" associated with medullary injury is shown to border an area of normal tubular architecture.

The histological findings in the presence of infection are characterized by collections of polymorphonuclear leukocytic exudate within and outside of the tubules and, in the affected portion of the cortex, the occurrence of necrosis and abscess formation. The entire acute process is illustrated in Figure 7, a low-power magnification.

TABLE 3. INITIAL GROWTH OF ORGANISMS

		Rabbit No.	*1*	*2*	*3*	*4*
Left	Upper pole (normal)	cortex	10^1	10^5	10^2	10^2
		medulla	10^2	10^6	10^4	10^2
	Lower pole (cautery injury)	medulla	10^5	10^7	10^6	10^2
		cortex	10^2	10^5	10^4	10^3
Right	Whole kidney		10^2	10^2	10^3	10^3

Bacteriological examination of kidneys 12 hours after intravenous injection of *E. coli* in rabbits with medullary injury on the left. The kidney was sectioned, as shown in Figure 8. Bacterial counts are expressed as colonies *E. coli*/100 mg. of kidney. Infection does not appear to have taken place in Rabbit No. 4.

Site of earliest bacterial growth. Experiments were designed to determine the site of initial bacterial multiplication in kidneys with medullary injury. After the usual interval of 7 days following cauterization of the medulla, the infecting dose of bacteria was given intravenously. Twelve hours later the kidney was removed, and four pieces of it were excised, weighed, and cultured quantitatively. One of these was from the medulla in the region of the cautery injury. A second was from the cortex tributary to the area of injury. The other two were from comparable sites at the uninjured pole of the kidney. Figure 8 illustrates these sites. The results of the first 4 experiments are given in Table 3. Apparently infection was not developing in one of the animals (No. 4). In the other three, however, the noteworthy finding is that the highest bacterial counts were found in the medulla in the region of the preceding injury, indicating that growth of bacteria either begins first or proceeds more rapidly there.

A second series of experiments was carried out to obtain further information on this point, shortening the time interval to 6 hours after the intravenous inoculation, and employing a different means of medullary injury which had been found to be associated with a similar effect on susceptibility. In this case, instead of a cautery injury, 0.1 ml. sterile saline was injected into the lower pole medulla, and the bacterial inoculum was injected intravenously at the conclusion of the operative procedure. Samples of kidney tissue, similar to those described in the preceding experiments were tested

TABLE 4. INITIAL GROWTH OF ORGANISMS (CONT'D)

		Rabbit No.	*1*	*2*	*3*	*4*
Left	Upper pole (normal)	cortex	10^4	10^2	10^1	10^2
		medulla	10^6	10^3	10^4	10^4
	Lower pole (saline injection)	medulla	10^6	10^6	10^5	10^6
		cortex	10^3	10^3	10^4	10^3
Right	Whole kidney		10^2	10^2	10^2	10^2

Bacteriological examination of kidneys 6 hours after intravenous injection of *E. coli* in rabbits with 0.1 ml. sterile saline injected into the lower pole medulla on the left. The kidney was sectioned, as shown in Figure 8. Bacterial counts are expressed as colonies *E. coli*/25 mg. kidney. **In Rabbit No. 1 a hematoma was found in the center of the medulla at the site of the injection. This may account for the large number of organisms found in the medulla of the upper pole.**

for bacterial content. The results are shown in Table 4. Again the findings indicate that bacterial multiplication begins first in the medulla near an area of injury.

Course of the infection. Because the main interest in these studies was on the factors which influence the inception of infection in the kidney, a systematic study of the outcome has not been made. Some animals were, however, maintained for 4-8 weeks after inoculation of the bacteria. The bacteriological findings in these are shown in Table 5. It will be observed that the infection had a tendency to "burn out," and that comparatively few bacteria were recovered, especially in the group allowed to go 8 weeks after infection. Of interest is the fact that the numbers of bacteria persisting in bladder urine were often greater than those found in the substance of the kidney.

The gross appearance of the lesions at 4-8 weeks was characterized by scarring and contraction in the affected zone. In some specimens there was hydronephrosis of the entire renal parenchyma, which is of interest in view of the fact that no primary injury or obstruction to the ureter had been done. Possible explanations are: (a) sloughing of necrotic tissue from the medulla may produce ureteral obstruction; (b) ureteritis may give rise to obstruc-

TABLE 5. CHRONIC INFECTION

Rabbit No.	*Time from injection of bacteria*	*Colonies E. coli*		
		Lt. kidney	*Rt. kidney*	*Bladder urine (per ml.)*
1	4 weeks	10^1	0	+
2	"	0	0	0
3	"	10^2	0	$>10^5$
4	"	$>10^7$	0	$>10^7$
5	"	0	0	0
6	"	10^4	0	+
7	6 weeks	10^3	0	10^7
8	"	0	0	0
9	"	0	0	0
10	"	10^2	0	$>10^5$
11	"	10^2	0	$>10^6$
12	"	0	0	0
13	"	10^3	0	10^7
14	8 weeks	10^1	0	0
15	"	0	0	0
16	"	0	0	0
17	"	0	0	0
18	"	0	0	0

Bacteriological examination of kidneys and bladder urine at 4, 6, and 8 weeks after the intravenous injection of *E. coli* into rabbits with cautery injury in the left renal medulla.

tion; or (c) previously uninvolved collecting ducts may be distorted by the formation of scar tissue around the original injury, with consequent retraction of the papilla and appearance of hydronephrosis.

Infection of the urine. In the course of many experiments, in rats as well as in rabbits, the experience in this laboratory has been that intravenously injected coliform bacilli are not excreted into the urine by normal kidneys. Cultures of bladder urine during the first 6 hours after the intravenous

inoculation of bacteria have not yielded *E. coli,* nor were they found in occasional tests at later periods.

In the presence of a medullary lesion, when infection is developing in the kidney, bacteria appear in the bladder urine soon after the intravenous injection. In a group of 7 animals with medullary lesions, examined 12 hours after the intravenous injection, positive cultures were obtained from

TABLE 6. INFECTION OF KIDNEY VIA BLADDER URINE

Rabbit No.	*Time from injection of bacteria*	*Colonies E. coli*	
		Lt. kidney	*Rt. kidney*
1	7 days	$>10^7$	0
2	"	0	0
3	"	0	0
4	"	10^2	0
5	"	10^6	0
6	"	0	0
7	"	$>10^5$	0
8	"	0	0
9	10 days	$>10^5$	$>10^3$
10	"	$>10^5$	0
11	"	0	0
12	"	0	0
13	"	$>10^5$	0
14	"	10^4	0
15	11 days	0	0
16	"	0	0
17	21 days	0	0
18	"	0	0
19	"	$>10^7$	0
20	"	$>10^5$	0

Bacteriological examination of kidneys following the inoculation of *E. coli* into the bladder of rabbits whose left renal medulla had been injured 7-10 days previously.

bladder urine in 5. Obviously then, in the presence of a medullary lesion, when infection is beginning to occur in the substance of the kidney, bacteria readily gain access to the renal pelvis and lower tract.

Introduction of bacteria by way of the bladder urine. An experiment was performed to determine whether kidneys with medullary injury would become infected if the bacterial inoculum were placed in the bladder urine instead of the blood stream. Cautery injuries were established in the usual

manner in the left kidneys of 20 rabbits, either in the papilla or elsewhere in the medulla. One week later the standard inoculum of bacterial culture was injected into the bladder cavity at laparotomy. After a further time interval of 1 or 3 weeks the kidneys were examined for presence of infection. The results are given in Table 6. It was found that large numbers of bacteria and gross appearance of infection had occurred in the left kidneys of 9 of the 20 animals. It appears, then, that in the presence of medullary injury, bacteria introduced into the lower urinary tract may succeed in establishing pyelonephritis which is in every way similar to that produced by intravenous inoculation of these organisms.

Occurrence of bacteremia in this form of pyelonephritis. Blood cultures were made on 38 animals with medullary scars at varying intervals after the intravenous inoculation of the colon bacilli. Bacteremia was demonstrated in 10 of 12 cases within 24 hours of the inoculation. This, however, is the usual finding in the case of normal animals given the same inoculum intravenously. Blood cultures were negative in the remaining 24 tests done between the second and seventh days, despite the fact that acute pyelonephritis was present in the majority of the animals.

Tests for occurrence of spontaneous infection in animals with medullary injury of the kidney. Twelve rabbits subjected to cautery injury of the medulla were not given an inoculation of bacteria by any route, and then were examined for evidence of development of infection in the injured kidney. The interval between injury and cultural examination was 10 days in 4, 4 weeks in 4, and 6 or 8 weeks in 2 each. Cultures of both kidneys and bladder urine were sterile in every case.

DISCUSSION

The findings in these experiments, in which small areas of injury were placed in selected areas of kidney substance, show an unmistakable difference between cortical and medullary tissue. Even when 10 to 20 lesions were placed in the cortex, there appeared to be no effect on susceptibility to infection with *E. coli* introduced via the hematogenous route, whereas a single lesion in the medulla caused infection to "take" in approximately 85 per cent of instances.

The different effect of cortical and medullary lesions makes it seem unlikely that the role of the cautery injury is simply the production of an avascular focus of necrotic tissue in which bacteria can multiply, protected from cellular or humoral defense mechanisms.

The fact that the medullary lesion obstructs the drainage of a group of nephrons brings up the possibility that prevention of excretion of bacteria from this portion of the kidney may be responsible for the development of infection there. This requires a review of the role of the kidney in the disposal of bacteria injected into the blood stream. It should be pointed out first that the kidney is not effective as a "trap" for bacteria which gain entrance to the circulation. In our previous study in rats[7] it was found that only about 1 in 10,000 of the organisms injected intravenously lodged in the kidney. The findings in the present studies indicate that the rabbit is comparable. Furthermore, as already described, no evidence has been obtained in these studies, or in those of some others,[1] that bacteria carried to the kidney in the blood are excreted via the tubules and the urinary passages, unless, as in the case of staphylococci, they are capable of setting up an infection in the normal kidney. It seems unlikely, therefore, that the effect of medullary lesions in enhancing susceptibility to infection is through prevention of excretion of bacteria by that segment of the kidney. On the other hand it is reasonable to suppose that once bacterial multiplication has begun, the spread of infection within the area is facilitated by the existence of obstruction of tubular drainage and the ability of organisms to multiply in the contents of these obstructed nephrons.

The view that medullary lesions enhance susceptibility to infection because they obstruct tubular drainage is difficult to deny. Several possible mechanisms for this suggest themselves. DeNavasquez was of the opinion that in the presence of "intrarenal hydronephrosis" there was a "slowing of the circulation and consequent trapping of the circulating bacteria."[8] This hypothesis seems unlikely to us. In previously reported studies[7] even in the presence of complete ureteral obstruction and severe hydronephrosis there was no significant increase in number of bacteria deposited in the kidney during the early phase of the bacteremia, as compared with the contralateral, unobstructed organ. Another possible mechanism is local increase in hydrostatic pressure. This could affect the inflammatory response to infection, diapedesis of leukocytes, or the lymphatic circulation in the area. Another factor which may have much to do with the relation between tubular obstruction and susceptibility to infection is the chemical alterations which must take place in such an area, inside the cells as well as in the fluids in and around the tubules.

The evidence obtained by culturing small pieces of kidney tissue early in the course of the infection, i.e., 6 to 12 hours, seems to indicate that growth of bacteria proceeds much more rapidly in medullary tissue than in the corresponding cortical area. Indeed, it seems possible that the cortical

abscesses are secondary to heavy initial growth in the medulla, that is, they develop as an extension of medullary infection. Experiments which may shed light on this possibility are in progress.

The results of the experiments in which bacteria were introduced into the bladder urine seem of special pertinence to the problem of pyelonephritis in man. There is good cause to support the belief that many, perhaps most, cases of human pyelonephritis are the result of infection which reaches the kidney from the lower urinary tract.[2] It appears from the present studies that bacteria growing in the bladder urine may be capable of reaching the kidney and establishing an infection there, provided that the kidney has been rendered susceptible, as by a lesion in the medulla. The pathway by which they travel to the kidney is not established; it could be by way of lymphatics, blood stream, or ureter. The important point to be stressed, however, is that infections developed in the kidney following inoculation of bacteria into bladder urine.

Perhaps related to the foregoing is the fact that occasional infections were encountered in the normal, contralateral kidney within a 2-week period following establishment of this form of experimental pyelonephritis. This too is in accord with observations of pyelonephritis in man, which often appears to begin in one kidney but tends to become bilateral eventually. In view of the rare demonstration of bacteremia and the usual occurrence of heavy bacteriuria in naturally-occurring or experimental pyelonephritis, it seems likely that infection of a kidney via upward spread of infection in the ureter is possible. Conceivably, the coliform bacterial infections of presumably normal kidneys observed in rare instances in these experiments were the result of heavy and continuous seeding of infection from elsewhere in the urinary tract. Unfortunately, gross evidence of this infection was not detected at the time the kidneys were removed, and the existence of large numbers of bacteria in them was only recognized when the results of the cultures were obtained. These are the only instances we have encountered of infection with this strain of *E. coli* in the uninjured kidney of the rabbit.

In the acute experiments described here one of the most impressive findings was the observation that acute pyogenic infection tended to remain confined to the area of the kidney affected by the medullary lesion, in spite of the fact that the urine in the pelvis of the kidney teemed with bacteria. In the acute stage, at any rate, there seems to be little tendency for unobstructed portions of the kidney to become infected, either by way of lateral spread in the kidney substance or from the pelvic urine.

The high degree of susceptibility to infection in the kidney with complete obstruction (due to ligation of the ureter) or with complete obstruction of a

segment (due to a medullary lesion) is of interest in connection with our results in animals with partial ureteral obstruction where infection developed in only about 1 of 5 instances[8] in spite of the fact that there was severe ureteral, calyceal, and tubular obstruction and stasis of urine flow. The explanation for this difference in susceptibility is not apparent at the present time.

SUMMARY

Coliform bacteria, although commonly associated with clinical pyelonephritis, are incapable of establishing pyelonephritis in experimental animals when injected intravenously and are not excreted into the urine. It was found that injury to the renal medulla of the rabbit followed by intravenous inoculation of *E. coli* led to acute pyelonephritis in approximately 85 per cent of instances, whereas similar lesions in the cortex were without that effect. Spontaneous development of infection in kidneys with medullary injury was not observed.

The infection established in a kidney after injury to the medulla is an acute pyogenic process which remains confined to a wedge-shaped area including that portion of cortex which excretes urine through the area of injury. There appears to be no tendency for it to spread into adjoining parts of the organ. The acute infection subsides after several weeks, leaving an area of extensive scarring. The bladder urine may contain large numbers of bacteria at a time when they are no longer demonstrable in the kidney.

Studies of the initial phases of this infection indicated that bacterial growth could be detected in the medulla near the area of injury earlier than in the corresponding part of the cortex. By the end of 24-36 hours all parts of the kidney substance tributary to the area of injury were heavily infected. The presence of organisms in the urine was detected within a few hours after the onset of the acute renal infection. Bacteremia, on the other hand, could not be demonstrated to persist longer than would be expected as a result of intravenous administration of these bacteria in normal animals.

It was also possible to produce kidney infection in the presence of medullary injury when organisms were introduced into the bladder urine. The incidence of positive results was about 50 per cent, and the lesions were similar to those resulting from infection by the intravenous route.

In rare instances there was evidence that heavy bacterial growth had taken place in the contralateral, uninjured kidney, some days after acute pyelonephritis, with its attendant bacteriuria, had been established in one kidney.

The means by which medullary injury increases the susceptibility of kidney tissue to bacterial infection is thought to be related to obstruction of collecting tubules, but the exact mechanism of this is not obvious.

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FROM ENDOTOXIN TO SNAKE VENOM**

The host-parasite relationship in many infectious diseases of animals and of man continues to challenge the knowledge and imagination, not only of microbiologists and those in other basic disciplines, but that of clinicians as well. There remain many unexplained features of this relationship bearing on the problem as to why invading microorganisms cause illness. One aspect of this relationship which has attracted considerable attention in recent years involves Gram-negative bacteria. The metabolic and morphological alterations induced in the host by bacterial somatic preparations known as endotoxins have been studied intensively in many laboratories and clinics. The purpose of this report is not to review the extensive literature on the subject but rather to summarize the results of investigations which have been carried out by my associates and myself. I shall refer largely to various communications from our group in the Department of Medicine and from those of Dr. Maurice Visscher and his associates in the Department of Physiology, which in turn document the related work of others.

WHY INVESTIGATE THE ACTION OF ENDOTOXINS?

My interest in endotoxin was aroused almost 25 years ago while working on the problem of gonococcal arthritis with Dr. Chester S. Keefer. During the course of those investigations it was observed that gonococci, in common with many other species of Gram-negative bacteria, were killed by human blood without the intervention of phagocytes.[30] In other words, fresh serum lysed the organisms. Further studies revealed that a suspension of lysed gonococci was extremely toxic when injected into the tissues of animals. In a discussion on the pathogenesis of gonococcal infections at the time, it was postulated that the inflammatory reaction induced by gonococci was due, in part at least, to the autolyzed organisms.[28]

With the advent of the sulfonamides and antibiotics the problem of gonococcal disease offered less of a clinical challenge, and interest in endotoxin subsided until attention was attracted to the possible role of endotoxin

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in the pathogenesis of illness caused by brucellae. While investigating the therapy of brucellosis in Mexico City with my associate, Dr. A. I. Braude, and with Dr. M. Ruiz-Castaneda, an interesting sequence of clinical events aroused a further interest in endotoxin.[27] Patients having severe and prolonged illness due to *Brucella melitensis* were given chlortetracycline. Shortly after the initial dose of the drug, the condition of several of the patients became worse. They exhibited a rise in temperature, tachycardia, and an alarming decline in blood pressure. Subsequently, they showed marked improvement. These manifestations suggested that the antibiotic shared in the destruction of large numbers of brucellae in the host with the liberation of endotoxin from the lysed organisms. It was postulated that the condition of the patients became worse not only because of the inherent toxicity of the endotoxin, but because they had acquired hypersensitivity to brucella antigen. This concept was to occupy our thoughts for several years.*

Further general interest in endotoxin was stimulated by clinical observations in patients having systemic infections due to several species of Gram-negative bacteria commonly associated with urinary tract infections. These microbes are not highly pathogenic but they do threaten life when they invade the blood stream, probably largely due to the endotoxin of the organisms. The endotoxin frequently participates in the syndrome of shock.[27] These clinical observations stimulated an extensive study on the hemodynamic changes induced by endotoxin in cooperation with the Department of Physiology.

INTRODUCTORY STUDIES WITH BRUCELLA ENDOTOXIN

Endotoxin has been described as a lipoprotein-carbohydrate complex localized on or near the surface of Gram-negative bacterial cells. Endotoxins have been prepared according to a variety of procedures. Elaborate techniques have been detailed for making "purified" material. Some have concluded that the toxic component in endotoxin is a macromolecular polysaccharide fraction. We believe that this concept of endotoxin is too narrow, although the carbohydrate moiety is an important toxic factor. It is not unlikely that the disintegration of bacterial cells in the host results in the liberation of several undefined, but active substances. The sum total

* In this presentation, I shall not develop this concept any further. This has been done elsewhere (Spink, W. W., *The nature of brucellosis*, University of Minnesota Press, 1956, Chap. 6; Spink, W. W., "The significance of bacterial hypersensitivity in human brucellosis: Studies on infection due to strain 19 *Brucella abortus*," *Ann. int. Med.*, 1957, *47*, 861; Abernathy, R. S. and Spink, W. W., "Studies with brucella endotoxin in humans: The significance of susceptibility to endotoxin in the pathogenesis of brucellosis," *J. clin. Invest.*, 1958, *37*, 219.

effect of these somatic components on the host also remains undefined. At the present time, when purified somatic bacterial material is presented to the host, a reproducible train of events ensues, but this does not necessarily reflect the course of changes under natural conditions, in which the host is challenged with many unknown bacterial factors.

Nevertheless, in order to reduce the number of variables to a minimum, we have also tried to employ a standardized and partially purified preparation of endotoxin. With but few exceptions we have used a Boivin-type of endotoxin, which involves extraction of the bacterial cells with trichloracetic acid. The method for making the endotoxin has been given in detail elsewhere.[29]

Many of our investigations with endotoxin have been carried out in mice. Again, this selection was carried out in an attempt to lend uniform conditions to the many experiments. Mice are quite suitable for comparative studies because a genetically homogeneous population was available, and, also, because relatively small amounts of endotoxin were necessary for experimental purposes. Our initial studies on brucella endotoxin in a population of male ABC mice compared the toxicity of endotoxin prepared from strains of brucellae having varying degrees of virulence. The toxicity of endotoxin had no relationship to the virulence of the living culture. Endotoxin prepared from a culture of Strain 19 of *Br. abortus,* having attenuated virulence, was just as lethal for mice as endotoxin made from a highly invasive strain of *Br. melitensis.*[28] Death of the mice uniformly occurred between 12 and 24 hours. More recent studies have shown that endotoxins prepared from *Escherichia coli* and *Salmonella typhosus* were weight for weight more toxic for mice than brucella endotoxin.

STUDIES IN THE RELATIONSHIP BETWEEN ADRENOCORTICAL ACTIVITY AND ENDOTOXIN

Having established that endotoxin from brucellae, as well as endotoxin prepared from other species of Gram-negative bacilli, was lethal for mice, a series of studies was made on the role of the adrenal cortex in the defense mechanism of the mouse against brucella endotoxin as well as against viable brucellae. Interest in this aspect of the endotoxin studies was stimulated by the report of others[30] stating that the generalized Shwartzman reaction induced by endotoxin in rabbits was greatly accelerated by administering cortisone. It was anticipated that cortisone would abet the lethal action of endotoxin in mice. On the contrary, it was demonstrated repeatedly that administering cortisone protected mice against lethal doses of endotoxin.[28]

The influence of endotoxin on the normal temperature rhythm of the mouse was also studied in relation to adrenal activity. First, the 24-hour rhythm of temperature in the normal mouse was determined. The rectal temperatures varied between 36° and 39.5° C., with the lowest temperatures occurring during midday and the highest readings in the early morning hours. The peak of the temperature corresponded with the nocturnal activities of mice. This activity, in turn, can be directly correlated with adrenal function. It was observed that endotoxin caused a rise in temperature within seven to nine hours after endotoxin was administered, and then a profound drop in temperature to 30° C. occurred shortly before death.[12] Very small quantities of endotoxin altered the physiological rhythm of the temperature without causing death. Adrenalectomized mice were particularly susceptible to the action of endotoxin, both with respect to lethality and to alterations in temperature. However, administered corticosteroid stabilized the temperature in intact and adrenalectomized mice given endotoxin, as well as protecting them against a lethal outcome.[11, 18]

Though not directly related to the problem of endotoxin activity, observations were made in mice which were *simultaneously* infected with brucellae and given cortisone.[8] Under these circumstances a relatively benign disease was converted into a fulminating infection with a marked proliferation of the brucellae in the tissues. Adrenocorticosteroid administration had little adverse effect on more chronically infected mice. These observations in mice could not be correlated with subsequent investigations in human patients. The differences observed between acute brucellosis in mice and in humans, and the response to steroids, could be ascribed to the fact that brucellae and steroids were given to the mice at the same time, whereas in man, invasion of the tissues by brucellae had occurred before the steroid was given. This permitted stimulation of the defense mechanism in the human host and the establishment of an immune response before exposure to exogenous steroid. Another possible explanation for the difference is the relatively large dose of steroid used in mice. Patients seriously ill with acute brucellosis were dramatically improved within a short period of time when given steroid.[20] These studies have resulted in the recommendation that steroid therapy over a brief period of time should be employed along with the antibiotics in the management of the more severely ill patients.

A series of studies has been directed by Melby and Egdahl[21] on the effect of endotoxin on activity of the adrenal gland in the dog. By direct cannulation of the adrenal vein the secretion of hydrocortisone was measured under different circumstances. Brucella or *E. coli* endotoxin administered intravenously to dogs resulted in a prompt rise in the output of hydrocortisone.

This effect was mediated through the pituitary gland, since administered endotoxin was without effect in hypophysectomized animals, although a rise in the level of hydrocortisone could be provoked in these animals with ACTH. This pointed out quite clearly that in the dog the effect of endotoxin on adrenal activity was mediated through the pituitary. Similar observations have been recorded by others[38] for the rat. Following the initial response of the adrenal to endotoxin in intact animals, a further response could be elicited by ACTH but not as marked as that obtained prior to the administration of endotoxin. It was concluded that although endotoxin caused an increased secretion of cortical hormone, the functional capacity of the cortex was temporarily diminished by the endotoxin.

These studies were extended to human patients having shock due to bacterial infections.[22] It was observed that the blood levels of hydrocortisone were considerably higher in the patients with shock than in normal controls. Of further interest was the finding that administered hydrocortisone disappeared more rapidly from the blood of the patients with shock than from the blood of normal persons. But this rapid metabolism of exogenous hydrocortisone uniformly took place only in the patients who recovered. In the patients dying of shock the half-life of administered hydrocortisone was more prolonged than in normal individuals and considerably more so than in patients with shock who recovered.

STUDIES ON THE HEMODYNAMIC CHANGES INDUCED BY ENDOTOXIN

In an attempt to acquire more precise information concerning the nature of shock induced by endotoxin a group of experimental studies was designed in cooperation with members of the Department of Physiology. When large doses of endotoxin prepared from either *E. coli* or *Br. melitensis* were injected intravenously into dogs, a two-stage decline in arterial blood pressure was observed.[39] Within 30 seconds following the administration of endotoxin there was a precipitous drop in the systemic arterial pressure, and a simultaneous rise in the pressure of the portal vein. As the portal vein pressure returned to the pre-injection level, a rise in the femoral arterial pressure took place. This was then followed by a gradual decline in the systemic pressure over a period of several hours ending in death of the animals. It was demonstrated quantitatively that the *immediate* drop in arterial pressure was due to the pooling of the blood in the liver and intestine, and a reduction in venous return to the heart.[39] This immediate effect on pressure could be abolished by eviscerating the animal. However, this did not prevent the secondary decline in blood pressure. A progressive fall in blood pressure occurred in the eviscerated dog when cardiac filling

was maintained at a constant level, which indicated a decline in total peripheral resistance. It is unlikely that in the dog the shock induced by endotoxin is mediated through the central nervous system.[25] It was concluded that the critical problem in the dog with endotoxin shock was the initial pooling of blood in venous beds. This results in a failure of venous blood to return to the heart, causing in turn a diminution in cardiac output.[24] However, it should be emphasized that a species difference has been observed in the response to endotoxin by Dr. L. Hinshaw and his group working in Dr. Visscher's laboratory. Immediate engorgement of the liver was not noted in the cat, rabbit and monkey, possibly because constriction of the hepatic vein does not occur in these species as in the dog. But a progressive fall in systemic blood pressure was seen similar to that in the eviscerated dog. Thus, there is no evidence in the cat, rabbit and monkey for pooling of blood in the splanchnic bed, but a gradual decline in peripheral resistance does occur.

The foregoing observations in the dog implied that if the arterial pressure was to be stabilized in endotoxin shock, the cardiac output must be increased. This could possibly be accomplished by decreasing the pooling of blood peripherally. Vasopressor agents were evaluated experimentally, and it was observed that metaraminol (Aramine) did diminish the venous pooling in the dog and increase the return of venous blood to the heart.[22] These observations were further extended to human patients in the treatment of shock.[21, 35]

The rapidity with which endotoxin altered the hemodynamics in the experimental animal and the apparent primary vascular effect of endotoxin prompted further quantitative studies employing different techniques. Using the method of MacLean *et al.*[30] for weighing the liver, direct observations on the dog's kidney were made in a similar way in the eviscerated dog.[34] As quickly as 15 seconds after the injection of *E. coli* endotoxin into the femoral vein there was a marked decrease in the weight of the kidney. In fact, the size of the kidney diminished under direct observation. The arterial pressure fell *after* the change in kidney weight, indicating that the vasoconstriction occurring in the kidney was not initially due to the decline in blood pressure. The effect of *E. coli* endotoxin on renal function was studied on the isolated and perfused kidney of the dog.[15] When the kidney was perfused with heparinized blood at a constant pressure from a heart-lung preparation, impairment of renal tubular function occurred. There was a prompt and progressive deterioration in the rate of para-amino hippurate clearance, and the specific gravity of the urine decreased markedly. Recent unpublished data by Hinshaw and others have shown that the changes in

renal function are probably related to the effect of endotoxin on the renal blood flow. The Tm PAH is not appreciably altered after endotoxin. When the influence of *E. coli* endotoxin on the pulmonary circulation was studied in the isolated heart-lung preparation of the dog and cat, the outstanding feature was an increase in small vein resistance with a gain in the weight of the lung.[16]

ACQUIRED RESISTANCE TO ENDOTOXIN

Endotoxin rapidly affects changes in the tonicity of the vascular system, which in turn contributes to further physiological and morphological alterations in the host. Many of these resultant abnormal factors can, and have been measured. A phenomenon of endotoxin activity that has been well confirmed in many laboratories is the development of tolerance or resistance of animals to endotoxin following the injection of repeated doses of the material. But, here again, the problem is complicated. Tolerance to the fever-promoting property of endotoxin is not synonymous with the development of tolerance against the lethal action of endotoxin. Recently, dogs have been made tolerant to endotoxin by Drs. Egdahl and Melby so that no fever was induced by endotoxin, but the same animals continued to manifest increased adrenocortical activity.

That aspect of the problem of resistance that has engaged our group more prominently has been related to the lethal activity of endotoxin. This interest was an outgrowth of the observation that ACTH and the adrenocorticosteroids protected animals against lethal doses of endotoxin. It was also observed that totally unrelated substances such as chlorpromazine, and the polymer, polyvinylpyrrolidone (PVP), protected mice against the lethal action of brucella endotoxin.[2,6] Protection has also been afforded by antihistaminics (Benadryl), yeast extracts, barbiturates (Nembutal), and an environment of lowered temperature.

One of the perplexing problems of endotoxin resistance that has been explored is the duration of resistance against a lethal dose of endotoxin following the injection of sublethal amounts.[1] In mice it was found that a single sublethal dose of brucella endotoxin offered homologous protection for as long as four months, and after three weekly injections of sublethal amounts, protection was extended as long as ten months. Heterologous protection against other endotoxin was of shorter duration. The precise nature of this form of immune response is not clearly understood. It was of further interest to demonstrate that mice challenged with a lethal dose of endotoxin but simultaneously given either adrenocorticosteroid or chlorpromazine were protected against subsequent lethal doses of endotoxin.[5]

Brucella endotoxin administered to mice also resulted in increased resistance to living brucella organisms.

ACQUIRED SENSITIVITY TO ENDOTOXIN

The preceding discussion has been concerned with the acquisition of increased resistance of mice to endotoxin. It was possible to alter the response of this species so that they became more susceptible to the lethal action of endotoxin. It has been generally confirmed that mice are relatively resistant to histamine, but an increased susceptibility, in some strains of mice at least, develops following injection with *Hemophilus pertussis* vaccine. Since the administration of endotoxin to the dog is followed by the liberation of histamine into the blood stream, it was of interest to see if pertussis vaccine increased the susceptibility of mice not only to histamine, but also to brucella endotoxin. A series of experiments in this direction has revealed further the complicated nature of the problem.[4] It was observed that pertussis vaccine increased the susceptibility of Swiss-Webster, but not ABC mice, to histamine. The injection of brucella endotoxin was not followed by increased susceptibility to histamine in either strain of mice. Both Swiss-Webster mice and ABC mice became more susceptible to brucella and to typhoid endotoxin after the administration of *H. pertussis* vaccine. And finally, the protective action of chlorpromazine and Benadryl against endotoxin was abolished after pertussis vaccination, but the protection offered by adrenocorticosteroids remained unaltered.

An increase in the susceptibility of mice to endotoxin was demonstrated in another way. Mice were infected with a small inoculum of *Br. melitensis.* Approximately seven days to four weeks later a dose of brucella endotoxin that was not lethal for uninfected mice was injected intraperitoneally into the infected animals, and death ensued within 24 hours. Repeated minimal injections of the endotoxin in the infected animals was associated with a marked inflammatory reaction in the visceral peritoneum. This was at first interpreted as a manifestation of specific brucella hypersensitivity. However, subsequent experiments showed that mice infected with brucella were also more susceptible to the endotoxin of *E. coli* than normal controls. This sensitizing effect of an infection to endotoxin has been noted by others,[5] who have concluded that an explosive multiplication of bacteria induced by the endotoxin was responsible for the accelerated lethal effect. Perhaps this affords an explanation for our own results, since we have been able to protect mice infected with brucellae against endotoxin by the simultaneous administration of chlortetracycline.

AND NOW — TO SNAKE VENOM

It became known to us that the train of events induced in animals by endotoxin could be produced by many other agents. Furthermore, the action of endotoxin simulates the manifestations associated with anaphylactic shock, although the latter involves a specific immunological mechanism. The term "anaphylactoid shock" has been employed in the past for the nonspecific reactions caused not only by endotoxin but by many colloidal preparations.[28]

Peptone is one of many substances that have been studied intensively in an attempt to elucidate the mechanism of anaphylactoid shock. We observed that a population of ABC mice were resistant to an injection of peptone, but peptone proved to be lethal to mice six days after the administration of *H. pertussis* vaccine.[17] In addition, mice sensitized to pertussis vaccine could be protected against the lethal action of peptone with cortisone, chlorpromazine, and an antihistaminic agent.

In comparing other substances with endotoxin we became particularly interested in snake venom because of the observations reported by Essex and his associates.[9] They stated that the administration of rattlesnake venom to the dog was followed by a prompt drop in systemic blood pressure, similar to that which we had observed following the injection of endotoxin. But instead of noting a rise in the weight of the liver, Essex and his group recorded a drop in the weight of this and other organs. Dr. Essex kindly supplied us with the rattlesnake venoms, *Crotalus terrificus* and *Cortalus atrox*. Studies in mice have been made with these and also with moccasin venom, *Agkistrodon piscivorus piscivorus*. Some preliminary studies have also been made with a tropical rattlesnake venom, *Crotalus terrificus terrificus* and also with a venom from *Bothrops jararaca*, supplied to us by the Butatan Institute in Brazil. We have observed in the dog that rattlesnake venom caused a prompt drop in blood pressure, but the pressure eventually became restored at normal levels. The gradual secondary decline in pressure seen with endotoxin did not take place. It has become quite apparent to us from observations made on mice that rattlesnake venom obtained from different snakes of the same species will produce varying results. This is attributable to the fact that snake venom is such a complicated biologic material.[7] In this respect the complexity of endotoxin simulates snake venom. It should also be appreciated by the investigator that venom obtained from the same snake at different periods of time will vary in biologic activity.

Direct observations on the vascular effects of snake venom have been made by Fulton *et al.*,[10] employing the everted cheek pouch of the golden hamster. Moccasin venom injected beneath the epithelium results shortly thereafter in a marked arteriolar constriction, stasis of the blood, and then the appearance

of petechiae, with a diapedesis of erythrocytes through the vessel walls at venous junctions.

Possible immunological relationships between snake venom and endotoxin have been explored just enough to invite further investigations. It has been observed in studies on the Shwartzman reaction that the administration of moccasin venom made animals refractory to endotoxin.[25] In the experiments cited rattlesnake venom was not effective. Others[39] noted some cross protection in animals between moccasin venom and the endotoxin of *Salmonella typhimurium*. We have found that populations of ABC mice can be protected against lethal doses of rattlesnake venom *(Crotalus terrificus)* following three weekly injections of brucella endotoxin. This resistance has endured for as long as 6 weeks after the last injection of endotoxin. Protection against such venom has also been demonstrated after the administration of *E. coli* and *S. typhi* endotoxins. The three endotoxins also gave good protection to mice against moccasin venom and the venom of *Bothrops jararaca*.

Further investigations are under way with the precipitin test, which has included the gel-diffusion technique, in an attempt to elucidate further the immunological relationship between endotoxin and snake venom. To date, with this technique it has not been possible to demonstrate cross precipitins in the sera of mice immunized with endotoxin or snake venom. Several well-defined precipitin bands could be demonstrated when antivenom horse serum and venoms were employed in the gel-diffusion method.

THE CRUX OF THE ENDOTOXIN PROBLEM

In attempting to formulate a basic concept concerning the mode of action of endotoxin we have looked upon endotoxin as promptly "triggering" alterations in vascular activity. Vasoconstriction, vasodilatation, and increased permeability of the vessels are rapidly followed by profound metabolic and morphological changes in the tissues of the host. Various substances such as norepinephrine, serotonin, histamine, heparin, and proteolytic enzymes are very likely liberated into the blood stream, and these in turn alter the physiological and metabolic equilibrium of the host. Endotoxin sets in motion a chain reaction within the tissues and body fluids of the host that may result in death.

The most outstanding feature of endotoxin activity is the rapidity with which endotoxin acts when introduced into either the circulation of the intact host or into the perfusate of isolated organs. Changes in vascular activity can be measured within 10 to 15 seconds after the administration of endotoxin. This would indicate that either endotoxin acts directly upon the

endothelial wall or that it causes an alteration in the blood, which in turn affects the function of the vessel. If the primary action of endotoxin occurs in the blood, the changes may involve enzymatic activity because of the almost immediate response induced by relatively small amounts of material.

There is some suggestive evidence that the primary action of endotoxin operates through some factor or factors in the blood, and then secondarily upon the tissues of the host, including the vascular endothelium. Rosenfeld[24] found that endotoxin prepared from *Pseudomonas aeruginosa, E. coli,* or *Serratia marcescens* inhibited the *in vitro* adrenocortical activity of the calf adrenal only when the perfusate consisted of homologous whole blood. No effect of endotoxin was elicited when artificial physiological perfusates were employed. Likewise, Hinshaw and his associates[18] working with the isolated and perfused lung of the dog, observed a pulmonary vascular response when heparinized blood was used as a perfusate, but not when dextran was employed. Also, there was little effect from endotoxin when defibrinated blood was used in the heart-lung-kidney preparation in place of heparinized blood.

The accumulating evidence emphasizes that the action of endotoxin is complex. Effects of endotoxin action must not be mistakenly offered as basic causes for the alterations induced in the host. Endotoxin "triggers off" a chain reaction of vascular and metabolic changes. The crux of the endotoxin problem in the host-parasite relationship resides largely in the few seconds that elapse after endotoxin appears in the blood stream of the host.

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of petechiae, with a diapedesis of erythrocytes through the vessel walls at venous junctions.

Possible immunological relationships between snake venom and endotoxin have been explored just enough to invite further investigations. It has been observed in studies on the Shwartzman reaction that the administration of moccasin venom made animals refractory to endotoxin.[25] In the experiments cited rattlesnake venom was not effective. Others[26] noted some cross protection in animals between moccasin venom and the endotoxin of *Salmonella typhimurium*. We have found that populations of ABC mice can be protected against lethal doses of rattlesnake venom *(Crotalus terrificus)* following three weekly injections of brucella endotoxin. This resistance has endured for as long as 6 weeks after the last injection of endotoxin. Protection against such venom has also been demonstrated after the administration of *E. coli* and *S. typhi* endotoxins. The three endotoxins also gave good protection to mice against moccasin venom and the venom of *Bothrops jararaca*.

Further investigations are under way with the precipitin test, which has included the gel-diffusion technique, in an attempt to elucidate further the immunological relationship between endotoxin and snake venom. To date, with this technique it has not been possible to demonstrate cross precipitins in the sera of mice immunized with endotoxin or snake venom. Several well-defined precipitin bands could be demonstrated when antivenom horse serum and venoms were employed in the gel-diffusion method.

THE CRUX OF THE ENDOTOXIN PROBLEM

In attempting to formulate a basic concept concerning the mode of action of endotoxin we have looked upon endotoxin as promptly "triggering" alterations in vascular activity. Vasoconstriction, vasodilatation, and increased permeability of the vessels are rapidly followed by profound metabolic and morphological changes in the tissues of the host. Various substances such as norepinephrine, serotonin, histamine, heparin, and proteolytic enzymes are very likely liberated into the blood stream, and these in turn alter the physiological and metabolic equilibrium of the host. Endotoxin sets in motion a chain reaction within the tissues and body fluids of the host that may result in death.

The most outstanding feature of endotoxin activity is the rapidity with which endotoxin acts when introduced into either the circulation of the intact host or into the perfusate of isolated organs. Changes in vascular activity can be measured within 10 to 15 seconds after the administration of endotoxin. This would indicate that either endotoxin acts directly upon the

endothelial wall or that it causes an alteration in the blood, which in turn affects the function of the vessel. If the primary action of endotoxin occurs in the blood, the changes may involve enzymatic activity because of the almost immediate response induced by relatively small amounts of material.

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The Board of Editors

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A COMPARATIVE STUDY OF THE CONCENTRATIONS OF NONPROTEIN, UREA, AND AMINO ACID NITROGEN IN THE MATERNAL AND FETAL PLASMAS OF SHEEP AND GOATS**

During the course of a study of placental function,[1] the concentration of nonprotein nitrogen in the fetal and maternal plasmas in sheep and goats was determined routinely. Although the samples were obtained during experiments which were primarily designed for other purposes, there was no reason to believe that the levels of N.P.N. would be altered. As the data indicated a difference in concentration on the two sides of the placental barrier, the study was extended to include a comparison of the urea and amino-acid nitrogen levels in the maternal and fetal plasmas as a step toward an understanding of the role of the five-layered, syndesmochorial placenta of the ruminants in the nitrogen metabolism of the fetus. The results of these comparisons, made on samples obtained from sheep and goats at known stages in the latter two-thirds of the gestation period (circa 145-150 days), form the substance of this report.

MATERIALS AND METHODS

The sheep and goats used in the experiments were healthy, well-nourished animals. The goats were either Toggenbergs or Sanaans, and the sheep, Dorsets or Hampshires. The animals were kept off feed for two days prior to the experiment. They were anesthetized with intravenous chloralose (7 ml. per kg. of body weight of an isotonic solution containing 5 g. of chloralose, 5.8 g. of sodium chloride, and 4.0 g. of sodium bicarbonate per liter of water).

The plan was to draw samples simultaneously from a fetal and maternal vessel into syringes moistened with heparin, but, frequently, one sample was obtained a minute or two after the other. The maternal samples were drawn from the uterine artery or vein, sometimes from both vessels, through catheters used to obtain the blood samples used for the estimation of uterine blood flow. The fetal samples were drawn through needles from either one or both of the umbilical vessels after delivery of the cord through a small incision in the uterine wall. When samples were drawn from both umbilical vessels and the uterine artery and vein, the results from each pair were averaged.

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Immediately after the samples were drawn, the cells and plasma were separated by centrifugation. The plasma was then removed and stored in capped syringes at 4° C. The nonprotein nitrogen was determined by the method of Wong, modified by Buell and Archibald as described in the manual "Clinical Methods for the Coleman Junior Clinical Spectrophotometer."[8] As the N.P.N. levels in the plasma samples were in some cases much higher than those normally occurring in human plasma, only 0.5 cc. samples were deproteinized with 9.5 cc. of trichloracetic acid so that the plasma was diluted 1-20 instead of 1-10 as in the original method. Digestion was carried out on 0.5 cc. portions of the filtrate. The urea and amino-acid nitrogens were determined in duplicate on 0.5 cc. samples by the methods of Van Slyke[10] and Hamilton and Van Slyke[9] respectively.

TABLE 1. TOTAL NONPROTEIN NITROGEN IN MATERNAL AND FETAL PLASMAS OF SHEEP AND GOATS (MGS. PER 100 ML. PLASMA)

Species	*Gestation age, days*	*Maternal plasma*	*Fetal plasma*	*Fetal maternal*
Goat	60	24.0	55.0	31.0
"	61	21.0	44.0	23.0
"	73	48.0	52.4	4.4
"	77	39.0	63.7	24.7
"	88	26.9	41.4	14.5
"	90	42.0	78.0	36.0
"	90	37.0	59.0	22.0
"	95	15.2	27.6	12.4
"	105	20.0	38.0	18.0
"	111	14.3	21.8	7.5
"	112	31.0	60.0	29.0
"	115	42.0	78.0	36.0
Sheep	115	49.7	84.7	35.0
Goat	116	37.0	64.0	27.0
"	117	39.1	53.4	14.3
"	120	58.4	73.6	15.2
"	122	19.9	34.0	14.1
"	123	63.6	58.9	—4.7
"	124	51.8	89.6	37.8
"	125	59.4	68.0	7.6
"	126	52.8	57.3	4.5
"	131	28.0	43.0	15.0
"	133	13.7	18.5	4.8
"	136	35.3	49.5	14.2
"	137	31.0	60.0	29.0
"	139	21.3	30.3	9.0
Sheep	141	25.0	26.0	1.0
"	142	29.0	49.0	20.0
"	144	17.0	33.0	16.0

RESULTS

Nonprotein nitrogen. Comparisons of the concentrations of the nonprotein nitrogen in the maternal and fetal plasmas were made in 25 goats and 4 sheep. The gestation ages of the fetal goats ranged from 60 to 139 days; the ages of the fetal sheep were 115, 141, 142, and 144 days respectively. The results of the comparisons are presented in Table 1. The concentration of the nonprotein nitrogen in the plasmas of the does and ewes varied over a wide range (i.e., 14 to 64 mgs. per cent); the range of

TABLE 2. UREA NITROGEN IN MILLIGRAMS PER 100 ML. OF PLASMA WATER

Fetal age in days	*Maternal plasma*	*Fetal plasma*	*Fetal minus maternal*
73	25.6	31.1	5.5
77	18.9	18.7	—0.2
78	19.7	18.7	—1.0
85	25.3	27.1	1.8
117	25.3	26.0	0.7
122	28.6	twin a 31.1	2.5
		" b 32.3	3.7
123	23.0	27.1	4.1
124	25.2	26.0	0.8
125	23.0	25.0	2.0
126	26.2	twin a 28.1	1.9
		" b 28.1	1.9
136	23.0	24.0	1.0
139	20.8	23.9	3.1

variation in the fetal plasmas was even greater—18 to 89 mgs.—with no apparent level, or change in level, associated with the gestation age in either the fetal or maternal plasma, though a high concentration in the maternal plasma was usually associated with a high fetal level. With but one exception —a goat with two fetuses 123 days' gestation age in which the fetal levels were, respectively, 1 and 3 mgs. per cent lower than the maternal—the nonprotein nitrogen concentration was higher in the fetal plasma by amounts that ranged from 1 to 38 mgs. per cent. That one exception is at the moment inexplicable, for there is nothing in our protocols to suggest that the circumstances of sampling or the condition of the doe were unusual.

Urea nitrogen. Comparisons of the urea nitrogen concentrations of maternal and fetal plasmas were made in 12 goats; two of the does carried twins. The fetal kids ranged in gestation age from 73 to 139 days. The results, corrected and expressed in mgs. per 100 cc. of plasma water (Table

2), demonstrate that the urea nitrogen of the maternal plasma varied between 18.9 and 28.6 mg. per 100 cc. H_2O while the range in the fetal plasma was between 18.7 and 32.3 with no apparent correlation between gestation age and plasma level. In two cases—in fetuses of 77 and 78 days' gestational age—the concentrations in the two plasmas were equal; in all the others the concentration of the urea nitrogen was higher in the fetal side of the placenta.

TABLE 3. AMINO-ACID NITROGEN IN MATERNAL AND FETAL PLASMAS OF SHEEP AND GOATS (MGS. PER 100 ML. PLASMA)

Species	*Fetal age in days*	*Maternal plasma*	*Fetal plasma*	*Fetal/Maternal*
Sheep	45	3.15	10.32	3.28
Goat	47	5.25	9.27	1.76
"	65	4.97	9.22	1.85
"	73	5.24	11.20	2.18
"	78	4.13	7.57	1.83
"	117	5.29	20.30	3.84
"	120	4.91	twin a 10.94	2.22
			" b 12.74	2.53
"	123	3.22	11.12	3.42
"	125	4.71	14.59	3.09
"	126	4.24	twin a 9.71	2.29
			" 8.99	2.12
"	136	6.21	15.13	2.44

The greatest difference in concentration across the placental barrier was 7 mgs. per 100 cc. plasma H_2O.

Amino-acid nitrogen. The concentration of the amino acid nitrogen was determined on the plasmas of 1 sheep, 8 goats, and their 13 fetuses. Though 5 of the does carried twins, plasma samples were obtained from both fetuses in but two cases. The results uncorrected for urea are presented in Table 3. In the plasma of the adults the concentration of the amino acid nitrogen varied from 3.22 and 6.21 with an average value of 4.67 mgs. per cent; the fetal levels ranged between 7.57 and 20.3, with the average 11.6. In every case the concentration was higher in the fetal than in the corresponding maternal plasma. The ratio of the fetal to the maternal concentration ranged from 1.76 to 3.84.

DISCUSSION

The data presented above, indicating that the concentration of the non-protein nitrogen in the plasma of the fetal kid or lamb tends to be higher throughout the last three months of the gestation period than it is in the

corresponding maternal plasma, are in general agreement with those of earlier studies of a similar character on whole blood, plasma or serum (see Needham's *Chemical embryology*, vol. 3, p. 1515). Direct comparisons are not easily made, for in the earlier work observations were either limited to materials obtained at the end of term,[11] or the gestation ages of the fetuses were not accurately known.[5, 6] Nevertheless, there appears to be agreement on two other trends between the observations reported here and earlier work: (i) the range of variation in the concentration of the N.P.N. in the fetal plasma or blood appears to be greater than it is in the maternal, and (ii) when the level in the maternal plasma is raised, the fetal level tends to be elevated as well.

Where comparisons have been made of the concentrations of urea in the maternal and fetal plasmas or bloods (see Needham[10]), the results indicate that in man, the rabbit, and the dog they are the same on the two sides of the placental barrier. In contrast, with but one exception (see Table 2) the urea concentration in the fetal plasma of the goat, expressed in mgs. per 100 cc. of plasma water, was higher than the maternal. As urea undoubtedly normally diffuses from the fetal to the maternal plasma, the tendency toward a steeper gradient in the goat may be related to the thickness of the tissue barrier that separates its fetal and maternal bloods. In the goat there are five separating layers, four in the dog, and three in man and the rabbit.[8]

There is general agreement among those who have used the method of Hamilton and Van Slyke[9] in their comparisons that the concentration of the amino-acid nitrogen is higher in the fetal plasma than it is in the maternal. Christensen and Streicher[3] found that the ratio $\frac{NH_2N_F}{NH_2N_m}$ was 1.7 to 1.8 in man, 1.5 to 2.0 in the rabbit, and 5.0 in the guinea pig. In samples taken at birth, Clementson and Churchman[4] found human ratios of the same order, i.e., 1.38-1.65; on similar material Crumpler, Dent, and Linden[7] reported ratios of 1.03 to 3.00. As the fetal-maternal ratios for the goats range from 1.76-3.84, the data available do not suggest that there is any correlation between the relative concentrations of amino acids on the two sides of the placental barrier and the number of tissue layers composing it for the ratios are of the same order of magnitude for man, rabbit, and the goat, though the placental barrier is formed by three tissue layers in man, a single layer in the full-term rabbit, and five in the goat.

The substantially higher concentration of the amino-acid nitrogen in the fetal plasma supports the suggestion that the amino acids are actively transferred by the placenta from the maternal to the fetal side; though it does not

establish that such a transfer takes place. The suggestion would, however, gain support if it were shown that maternal blood lost and fetal blood gained amino-acid nitrogen in passage through the placenta. This possibility is being tested.

SUMMARY

Comparisons have been made of the nonprotein, urea, and amino acid nitrogen concentrations in fetal and maternal plasmas of sheep and goats sacrificed at selected stages in gestation. In only one of 29 cases was the total N.P.N. of the fetal plasma lower than that of the maternal; in all others it was higher by varying amounts up to 37.8 mgs. per cent. The urea nitrogen, expressed in mgs. per cent of plasma water, was slightly lower in the fetal than in the maternal plasma in 2 of the 12 cases compared. In the other 10, it was higher on the fetal side, though the differences were small. The amino-acid nitrogen concentration in the fetal plasma was the higher in all 11 cases compared. The ratio of the fetal to the maternal concentrations ranged from 1.76 to 3.84.

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HERBERT THOMS* | *New Haven, Connecticut*

CHANNING AND SIMPSON IN EDINBURGH

In August of 1852 Dr. Walter Channing, professor of obstetrics and medical jurisprudence at Harvard Medical School, was about to finish an extensive tour of Europe, having left Boston early in May of that year. He was sixty-six at the time, and almost a half century had passed since his previous trip to that continent where as a medical student he studied in Edinburgh and London. The trip had taken him as far east as Moscow and St. Petersburg, north to Denmark, and south to Spain. On August 9th he was again in London, a city greatly changed over the years, "streets, squares and bridges —yes, London Bridge was not in its place. It had floated off down stream."

Channing's present destination was Edinburgh where he intended to catch his breath before going on to other parts of Britain, including Ireland. At Edinburgh the crowning event of his trip was to take place—a meeting with Professor James Y. Simpson, the first to use chloroform in childbirth. On separate continents and practically alone, these two great champions of the use of anesthesia in childbirth had each fought a bitter battle against prejudice and bigotry, a battle now all but won. They had much to say to each other.

At the time of Channing's visit Simpson was forty-one and at the height of his popularity and fame, although his baronetcy was a few years off and the most important single event since the discovery of anesthesia—the administration of chloroform to Queen Victoria during the birth of her fourth child, Prince Leopold—was six months away. For the women of Britain, this settled the matter for all time.

Their memorable meeting is described to some extent by Channing in a book called *A Physician's Vacation,* published in 1856. It is a travel classic of the period, the only flaw in its excellence being the author's flare for anonymity. Investigation, however, sometimes aided by 'slips' of the author himself, has filled in many of these omissions.

Walter Channing has numerous claims to present-day interest. He was Harvard's first medical dean, he was the first to describe the disease called

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pregnancy anemia, he was primarily responsible for the founding of the Boston Lying-In Hospital, he was a noted Shakespearean scholar, and he was the father of Ellery Channing, poet and intimate of Thoreau. But his greatest claim rests in his single-handed role in introducing anesthesia in childbirth in this country. Channing in America and Simpson in Europe are the two names always associated in the struggle for the general adoption of this means of reducing suffering in childbirth.

Except that he was born in Newport, Rhode Island, Channing had all the attributes of a Boston Brahmin. Both sides of his family had the "proper" background, his father being an eminent attorney and his mother a daughter of one of the signers of the Declaration of Independence, William Ellery. He was one of three distinguished brothers, all graduates of Harvard. One was the Reverend William Ellery Channing, the great Apostle ot Unitarianism and famous preacher of his time. The other was Edward T. Channing who influenced generations of students as professor of rhetoric at Harvard. There was one slight stain on Walter's Harvard escutcheon—his failure to receive his bachelor's degree on time because of his involvement in the student riot of 1807, but this was erased by the authorities a half century later, and the degree was granted "as of the Class of 1808."

On August 12, 1852 Channing was in Edinburgh gazing at Steedman's Lodgings on College Street where he had lived as a student. Changes had taken place here, too, but looking upward to the famous castle he says he felt at home. Channing took rooms at Gibbs' on Princes Street and after an early dinner went out for a stroll which ended at 52 Queen Street, Simpson's residence. Here he left his card and a letter. Within an hour of his return to the hotel his landlord appeared at the door with a companion who was introduced as Professor Simpson. Simpson insisted that Channing accompany him at once, his carriage being at the door. Channing, considerably overwhelmed, asked Gibbs what he should do and was informed that in Edinburgh everyone did pretty much as the Professor said. Within a matter of minutes Channing was in residence at No. 52 Queen Street, bag and baggage. This was characteristic of Simpson who was used to carrying everything before him. From this time on he took complete charge of Channing who remained his house guest for a month, forgetting Ireland and other plans.

Channing's account of his visit to Simpson is important to us, for here for the first time we see Simpson through the eyes of an American doctor. It is probable that no other guest of the great man ever saw him at closer range or over so extended a period. Channing saw patients with him, made

professional calls, went on excursions, attended social gatherings, and shared his family life. His description of Simpson's professional activities, fantastic as they appear to us, is corroborated by others. Simpson's office consultations, which lasted all afternoon until the dinner hour, brought crowds of patients filling many rooms; fifty and sixty patients in an afternoon was nothing rare.

As these men and women arrived, often in the morning, they were separated by an assistant into 'pay' and 'non-pay' groups, and there were many of the latter. Each member of a group drew a ticket for priority and each entered the consulting room as his number was called. Simpson's medical assistant took dictation, wrote prescriptions, and aided in the examination. Channing spoke of these sessions as home-clinics and wrote that he was learning much that would aid him in future practice. In the field of women's diseases Simpson was probably the foremost clinician of his time. He is often referred to as the father of gynecology. Under his guidance Channing was seeing a variety of cases not possible in the restricted clinics and hospital facilities of his own country.

Simpson liked Americans and American ways. It was the discovery of ether which had set him off on the series of investigations aimed to find a less irritating anesthetic. Although Channing was much older, Simpson was undoubtedly attracted to his guest, a polished gentleman with a fine sense of humor. It is interesting to note that both enjoyed amateur theatricals and that charades in the Simpson household was a popular game. Channing was young at sixty-six and always remained so.

Channing gives us this picture of his host.

> He is short, stout with small feet, and his step is short and very quick. I have to run somewhat not to lose him. Let me finish his picture. You have his length but not his full length. His head is large, covered with a profusion of black hair, which obeys its instincts, and more strikingly so when he thrusts his very small hand into and all over it. His forehead is of good height, but the hair grows low upon it; and to me this is the most becoming manner of its growth. . . . His eyes are singularly loquacious and always begin to talk before he utters a word. His knowledge is more various than I have before met with. Nothing escapes him. Science and literature are his pleasures. Archaeology is a favorite pursuit; and his friends frequently send him books ,and specimens which help his studies.

Channing continues:

> He receives a great deal of money, I have heard. But he seems wholly regardless of money, and as I have further heard, it is only lately that he has begun to accumulate property. He is paid at the visit, or consultation, which saves him from one of the most inconvenient offices; charging and collecting fees. It is when the patient is leaving

him, and by offering his hand for farewell, the fee is deposited in his. We feel both the inconvenience and the loss in America. I have seen fees paid him. I really think that if he were subjected to our system he would get no money at all. . . . I said his meals were often interrupted. His butler brings in cards, notes, letters; "There," says he and lays by note after note. Then two or three ladies come in. If he be not in, down they sit on the sofa, and take up books or newspapers. Then gentlemen, with or without ladies, appear. They are always asked to table by Miss ———, his sister-in-law, or by someone else. When the Professor is at table he places them. But he is reading or eating—or giving bread to a spotted Danish coach-dog named Billy, of fine size and an unusual pet. I feed him always. Professor Simpson talks to the comers. Thus he learns of strangers what they want, gets their residence, if visits are wanted, or goes into a room hardby and sees them alone. His house is very large, and full of rooms—and always seems inhabited.

Laing-Gordon, Simpson's biographer, quotes an observer at the noon-day table:

Lords and Commons rub shoulders at his table; the salt of the earth sit down side by side with the savourless; tweed jostles broadcloth; the town Briton looks askance at his country-bred compatriot, and both unconsciously shudder at the Briton with no breeding at all. In one room are assembled together the American of the bluest blood; the Yankee bagman; the slave-holding Southerner, and even the man of colour hateful to both alike. . . . If in one short hour Simpson could thus transform a crowd of frigid haughty strangers into an assemblage of decent amiable beings, what could he not achieve in a day, a year, or a life?

Channing had hardly unpacked his bags at No. 52 when the Professor had asked him to accompany him on a call which kept them up till midnight. At breakfast the next morning Channing found that his host had been called out again in the night. But this did not deter them from setting out on a week-end excursion to the Scottish Highlands. This was accomplished by rail to Glasgow, fifty miles; open boat to Ellensburg, six miles; coach to Luss, nine miles; steamer to Tarbit on Loch Lomond where they spent the night—in all, about a hundred miles. At the hotel as soon as it was found out that Simpson was in the house he was asked to see the proprietor's daughter who was ailing and, later on, to treat the proprietor himself. Channing was asked to consult on both cases, the result being that no bill was presented to either in the morning. Simpson reminded him that this was the first money he had earned since leaving America. Before retiring for the night they had strolled by the lakeside and after eleven had gone to bed, "like prudent men after a hard day's work." No doubt Channing was ready for bed. The night previous to his arrival in Edinburgh was spent in its entirety on the train from London.

After staying the night at Tarbit they drove a few miles to Arrochar on the northern end of Loch Long. Here they were the guests of Mr. McVicars,

a retired gentleman who had made a fortune in China. Of the guests Channing writes: "Our party was a rare one. It consisted of medical men all attached to Edinburgh University but one who is of the London University." He enumerates five professors who climbed aboard the host's carriage as they bade farewell the next day. If Channing included himself in the five, the others but one can be identified. They would be Simpson, Robert Christison, William Sharpey, and one other. Christison, later Sir Robert and world renowned as a toxicologist, was professor of materia medica and therapeutics at Edinburgh. Previously he had been professor of medical jurisprudence and in 1828 was important witness in the famous trial of Burke and Hare. Sharpey occupied the chair of anatomy and physiology at London. It is possible that the fifth was the surgeon James Syme, a close friend of Sharpey's and his active correspondent over a lifetime. Later, Channing was to see a good deal of Syme.

Channing was properly impressed by the McVicars' estate, the hedges, fruit trees, flower gardens, conservatory, the Elizabethan-type house. To him McVicars was the perfect Highlander with his bonnet and button. His lady, too, delighted him. Although Simpson and Channing had had breakfast at Tarbit, their host ordered another after which a climb of Fairy Mountain was proposed. Channing, and presumably McVicars, begged off from this adventure and spent the day walking over the grounds and watched the progress of the others through a telescope. The dinner and evening conversation made a particular impression on Channing. The next day the party was over and they journeyed back to Edinburgh, except Sharpey, who left them at Hamilton.

The Highland excursion opened the way for other hospitalities in Edinburgh. Christison entertained Channing at dinner, apparently a large one, for the "company embraced many of the distinguished men of Edinburgh." In his teaching of medical jurisprudence Channing had long been familiar with his host's work in this field. He was likewise greatly interested in Christison's studies of the geology of the Highlands.

Channing was also entertained by the surgeon James Syme whose operations he had witnessed at the hospital. Syme lived at Millbank near Edinburgh. As Crown Professor of Clinical Surgery he, too, attracted many visitors to Edinburgh. John Brown in his preface to *Spare Hours,* which contains the immortal "Rab and His Friends," gives a short but meaningful picture of Syme whom he served as medical apprentice, Syme being the surgeon in this finest of dog stories. Syme was no especial friend of Simpson for they were at odds with each other on many occasions. Syme had been

the foremost of those who opposed Simpson's election to the chair of midwifery. However, any real feeling between them was eventually dissipated when Syme became Simpson's physician during a serious illness and again when Simpson defended Syme after he was publicly attacked by an English surgeon.

Syme embodied much that would have attracted Channing. He was a superb surgeon who, says Brown, never wasted a word, a drop of ink, or a drop of blood. He was an expert amateur horticulturist interested especially in growing orchids.

At one of Simpson's dinners Channing met the principal or president of Edinburgh University—John Lee, then fifty-one years old, who first studied medicine, receiving his M.D. in 1801, and later entered the ministry, beginning his career as a parish clergyman. Before his appointment at Edinburgh he had been professor of moral philosophy at Aberdeen and at St. Andrews. Channing was much taken with Principal Lee, "whose manners were quiet, grave without dullness."

As has been noted, for the greater part of Channing's visit, he was figuratively Simpson's alter ego both in professional and private life. He was quick to view his celebrated host with the eye of a Boswell. One night on their way to bed, Simpson showed him his small bedroom which he referred to as his study. At the head of the bed was a bookcase filled with books. There was also a moveable gaslight for reading in bed. Channing was "surprised that he should make his study of his bed; and yet this simple incident had in it an explanation of the wide knowledge which is acquired under difficulties." While driving about they would discuss the cases they had seen together and often they would stop and visit some historic spot for a few minutes. They went to a number of places outside of the city, on one occasion to Carberry Hill where Queen Mary had surrendered to her insurgent subjects, another time to Pinkie House, a castle intimately associated with important events in Scottish history. These excursions were, however, usually sandwiched in between clinical pursuits, seeing patients at the home-clinic, and making professional outside calls. On the day before leaving Edinburgh Channing records that about ninety persons were at Simpson's house, among them the Duchess of Devonshire, a name he was unable to resist mentioning.

At six in the morning of September 15th he and Simpson breakfasted together and at seven left for the station where Channing took the train for Liverpool. Simpson "waited until the last bell" before saying his final good-

bye. Three days later Channing, with 141 other passengers, set sail on the steamer *Canada* for America.

Perhaps the most interesting thing about Channing's visit was the event itself. It was an occasion when two important leaders in medicine working in the same field had the opportunity of close association. In this instance it is more than likely that much of their talk was concerned with the opposition each had faced in the bitter fight to establish anesthesia in childbirth. No innovation in medicine ever aroused so much antagonism among the clergy, the laity, and the profession itself. The religious aspect centered in the primeval curse laid upon woman in Genesis 3:16 which reads in part, "Unto the woman he said . . . in sorrow thou shalt bring forth children." Simpson's study of the original Hebrew text enabled him to point out that sorrow really should be translated toil or physical exertion, as seen in the text, "in sorrow shalt thou eat of it all the days of thy life."* The Bible also presented Simpson with the most powerful argument of all in Genesis 2:21 which reads: "And the Lord God caused a deep sleep to fall upon Adam; and he slept; and He took one of his ribs and closed up the flesh instead thereof." Most theologians with whom Simpson had to deal found that his knowledge of Holy Writ was greater than their own. It is pleasant to record that Thomas Chalmers, the great Scottish divine, stated that the question of anethesia had no theological aspect and advised Simpson to take no notice of "small theologians" who opposed its use.

The main objection of the medical opponents was contained in the thesis that pain in childbirth was "a desirable, salutory, and conservative manifestation of life force." There were even extremists who maintained that anesthesia caused convulsions, paralysis, and even mental derangement. Simpson and Channing by published clinical studies demonstrated what is well known today, that anesthetic agents by diminishing excessive pain oftentimes shorten labor and facilitate delivery.

Channing's most important contribution was his *Treatise on Etherization in Childbirth,* of which he said; "It treats of a noble subject—the remedy of pain." In addition to his argument he published the results of anesthesia in 581 cases of childbirth.

Channing and Simpson never saw each other after bidding goodbye in 1852. Simpson died at fifty-nine in 1870. In a corner of St. Andrew Chapel,

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*** An interesting sidelight on this question presented itself in 1957 when it was proposed to introduce into the General Assembly of the International Federation of Gynecology and Obstetrics, which meets in 1958, a resolution which would give general endorsement to Simpson's interpretation of Genesis 3:16.**

Westminster Abbey, near the memorial to Sir Humphry Davy and other scientists, stands his striking bust. Underneath are these words:

To whose genius and benevolence
The world owes the blessings derived
From the use of chloroform for
The relief of suffering.

LAUS DEO

Channing lived for six years after Simpson's death, preserving to an advanced age his youthful spirit.

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EVENTS

Yale Medical Society

February 5, 1958

Experimental Induction of Ovulation. By Miriam E. Simpson, Department of Anatomy, University of California, Berkeley, and G. van Wagenen, Department of Obstetrics and Gynecology, Yale University School of Medicine.

The primate ovary can be stimulated readily and follicular growth will result. It has been the common experience, however, that ovulation with true corpus luteum formation follows only sporadically after the injection of gonadotrophins. Crude pituitary extracts from pig and sheep, purified follicle-stimulating hormone, equine serum gonadotrophin, and human chorionic gonadotrophin have all been tried, alone and in combination, with the same unsuccessful results.

Another effort has been made to determine the conditions essential for ovulation in the monkey *(Macaca mulatta)* in terms of the absolute amounts and proportions of the gonadotrophins necessary. Ovulation followed occasionally after injection of sheep follicle-stimulating hormone with or without supplements of luteinizing substance (pituitary interstitial cell-stimulating hormone or human chorionic gonadotrophin). However, when the extract was made from monkey rather than sheep pituitaries, ovulation followed dependably in both immature and mature monkeys. From both types of treatment a single ovulation resulted in the young animal and multiple ovulation in the adult.

The dosage of monkey pituitary needed for ovulation was considerably smaller than of the sheep anterior pituitary preparations. A total dose of 30 to 50 mg., given over 8 to 10 days, of a 40 per cent alcohol extract of monkey anterior pituitary (or the equivalent of about one pituitary daily) was adequate to induce ovulation when given to an adult female from the fifth day of the menstrual cycle. In terms of hypophysectomized rat units this was 1/20 to 1/30 the dose required of purified sheep follicle-stimulating hormone to induce ovulation.

M. E. S. AND G. VAN W.

Zoological Journal Club

February 25, 1958

Mechanism of Gene Duplication. By Franklin W. Stahl, Norman W. Church Laboratory of Chemical Biology, California Institute of Technology, Pasadena.

Ultracentrifugal studies of DNA from *E. coli* give further support, on the molecular level, for the postulated mechanism of duplication based on the Watson-Crick model of a two-stranded helix.

The method used is based on the fact that an 8 Molar cesium chloride solution in the ultracentrifuge cell produces a density gradient such that N^{15} labelled DNA and unlabelled DNA form distinct, separate equilibrium bands. The bacteria were grown for approximately twenty generations on a

nitrogen source of N^{15} labelled NH_4Cl; the first sample was then taken for DNA extraction. Immediately thereafter, the bacteria were transferred to unlabelled NH_4Cl, and further samples were taken at successive generations. Previous to the addition of unlabelled NH_4Cl, a single band was formed, corresponding to completely labelled DNA. One generation after the addition of unlabelled NH_4Cl, there was a single band at a point intermediate between labelled and unlabelled, indicating the presence of half-labelled DNA. After two or more generations there were two bands, one intermediate and the other completely unlabelled.

It has not yet been fully verified that the molecule being centrifuged is actually a single double helix. Nevertheless, the results are just those which would be predicted on the basis of the Watson-Crick model, i.e., that the DNA molecule exists in two subunits which separate during duplication, one subunit persisting in each daughter molecule.

F. M. WILLIAMS

ZOOLOGICAL JOURNAL CLUB

February 26, 1958

ACTIVELY ACQUIRED TOLERANCE OF HOMOLOGOUS CELLS. By Rupert E. Billingham, Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania.

A laboratory solution to the problem of skin homograft intolerance involves postnatal intravenous inoculation with whole cells. One-day-old mice of strain A inoculated with 5-10 million spleen cells from strain CBA into the sigmoid sinus show 100 per cent tolerance when later challenged with CBA skin grafts. Inocula reaching the lymphatic system fastest are most effective. If the inoculum is intraperitoneal, the tolerance is only 60 per cent; subcutaneous inocula are ineffective. A rapid fall in tolerance occurs if the inoculum is performed later: from 100 per cent within the first 24 hours, tolerance drops off sharply until it is absent after the fourth day. When seven days old, mice assume the adult immunization responses. This time schedule varies in different animals: rabbits are past toleration at birth, newly hatched chicks are at the threshold of immunization, but tolerance can still be induced in rats at 10 days. Chick embryos *in ova* can be injected with embryonic blood from another strain (injected adult blood kills the embryo) or two embryos may be parabiosed through the chorioallantois to give a homograft tolerance reaction.

Two genetically different types of cells may be found in spleen, liver, kidney, and thymus, the lymphatic system, and the leucocytes of such parabiotic twins, or of inoculated mice which show the tolerance reaction. Natural chimaerae of this sort have been found in dizygotic chicks from double yolk eggs and in three dizygotic pairs of human twins in Britain. Tolerance is only induced when nucleated cells are transferred; blood plasma and even avian erythrocytes are ineffective.

The closer two strains are genetically, the more easily tolerance may be brought about; earlier inoculation may compensate for genetic remoteness. Data from heterograft experiments tend to confirm this prediction. Post-

natally induced chick-duckling tolerance is impossible, and is even low in embryonic parabiosis. The turkey chick gives a fair tolerance reaction with the closely related chicken and pheasant. If adult antibody-producing cells are inoculated into a newborn host when its tissue is not ready to react to produce antibodies, the injected adult cells may produce antibodies which react againt host tissue, and result in abnormalities such as runt mice with involuted lymph nodes.

G. E. W. 3RD

YALE MEDICAL SOCIETY

March 10, 1958

THE EFFECT OF THEOPHYLLINE ETHYLENE DIAMINE ON THE PULMONARY CIRCULATION. By Ole Storstein, Department of Internal Medicine, Yale University School of Medicine.

In a group of 21 patients with various heart and lung diseases, exclusive of hypertensive heart disease, a study of the ventilatory and circulatory effect of theophylline ethylene diamine has been carried out. Theophylline ethylline diamine gave a distinct increase in maximal breathing capacity, indicating a bronchodilating effect. The improved ventilation lowered the arterial pCO_2, while the arterial O_2 saturation was unchanged. TED increased the heart rate and decreased the blood pressure both in the systemic and in the pulmonary circulation, but more pronounced in the latter. The cardiac output and the pulmonary capillary venous pressure were unchanged, and there was a pronounced fall both in the peripheral arterial and in pulmonary arteriolar resistance.

It is concluded that this pressure fall was mediated through a direct dilating effect of TED on the arterioles, both of the pulmonary and systemic circulations. The work of the right ventricle showed a fall which indicated that TED in this group of patients lessened the burden of the load placed on the right ventricle by pulmonary hypertension.

Compared to earlier investigations there seems to be a different reaction to TED among this patient group and patients with hypertensive cardiovascular disease. In the latter group the most pronounced effect was the increase in the cardiac output, while the vasodilating action on the pulmonary circulation was insignificant.

OLE STORSTEIN

MICROBIOLOGY SEMINAR

March 14, 1958

PHYSIOLOGICAL ACTION OF FUNGICIDES. By Saul Rich, The Connecticut Agricultural Experiment Station, New Haven, Connecticut.

Effective fungicides have been found by empirically testing thousands of compounds. The active compounds have been studied by many laboratories in attempts to elucidate mechanisms of fungitoxic action.

In a study by Owens, four groups of fungicides—the dithiocarbamates, phenols, quinones, and metals—were tested for their ability to inhibit amino, sulfhydryl, iron, and copper dependent enzyme systems in relation to their fungitoxic activity. The results showed few regular or predictable correlations, indicating that more than mere enzyme inhibition is involved.

Studies by Rich and Horsfall, and others, showed that permeation plays an important role. Thus the lengthening of an alkyl side chain progressively increases the activity of certain compounds, an effect which is correlated with increasing oil solubility. Other workers have shown that the toxicity of analogues of 8-quinolinol is related not only to the ability of these compounds to chelate but also to their degree of oil solubility.

The successful fungicide itself may not be the compound exerting fungitoxicity, but may instead break down to release the toxic material. Thus nabam, sodium *N*-methyldithiocarbamate, *N*-substituted rhodanines, and certain thiadiazines all break down to release highly fungitoxic isothiocyanates. Fungi may actually contribute to their own self-destruction. For example, Lukens and Sisler found that captan is rapidly broken down by sulfhydryl groups of the microorganism to release the highly toxic thiophosgene. Similarly, Byrde and his co-workers have demonstrated the ability of the fungus (as opposed to the host plant) to de-esterify the diacetyl ester of 2,3-dichloro-1,4-naphthoquinone resulting in the self-destruction of the fungus. This latter phenomenon is important because the parent quinone may be toxic to the host plant, whereas the ester is not.

Fungi may not only make compounds more toxic, but they may also detoxify other compounds.

It should be pointed out that the phenomenon of resistance as seen in antibacterial and anticancerous agents does not develop, in most instances, with fungicides.

S. I. H.

Cardiovascular Study Unit

Dextrocardia and Dextroversion in Congenital Heart Disease. By Robert P. Grant, National Heart Institute, Washington, D. C.

A right-lying heart may be manifested in three ways: first, as acquired dextroposition due agenesis of a lung or eventration of a diaphragm, etc.; second as congenital dextrocardia of the mirror-image type in which there is usually no cardiac abnormality other than position. This is always associated with situs inversus of the abdominal viscera; third as congenital dextroversion in which the heart lies on the right, as if turned like a page in a book, with the atria remaining in normal position.

Importance is attached to dextroversion because of the many structural abnormalities associated with it. With regard to the heart itself, cor biloculare, single ventricle, truncus, and other conditions are seen suggesting an interruption in development. Ninety per cent of these cases have transposition of the great vessels and interventricular septal defect. Arrest is believed to occur at a stage when the ventricular loop is largely on the right before the leftward migration of the bulbis, and this is the same fetal time when

differentiation of the conus and the truncus takes place. It is interesting that the splenic bulb appears at this time, because dextroversion is often associated with absent spleen and its manifestations such as target cells and erythroblasts in the peripheral blood. In contrast to situs inversus, in the abdominal heterotaxy seen in dextroversion the organs tend to be symmetric and primitive, as if arrested prior to the stage when the body first searches out its normal asymmetries and lateralities.

Diagnosis of dextroversion is made with the aid of a combination of x-ray, angiocardiography, and the electrocardiogram. Differentiation from other types of dextrocardia can be made from the ECG because in dextroversion the P waves in the conventional limb leads are upright while in mirror image dextrocardia they are inverted; on x-ray, transposition of the great vessels will be seen in many cases of dextroversion but not in mirror-image dextrocardia; on angiocardiography the normally laced atria and the lie of the right ventricle superior to the left will be noted in dextroversion, while in mirror-image dextrocardia the atria are reversed in location and the right ventricle is anterior to the left.

Similar diagnostic methods and reasoning may be applied to mesocardia and levocardia.

J. A. O'N.

EXPERIMENTAL DESIGNS. By William G. Cochran and Gertrude M. Cox. New York, John Wiley and Sons, Inc.; London, Chapman and Hall, Limited, 1957. 2d ed. xiv, 611 pp. $10.25.

This is a revision of a book which has already become established as an authoritative text on the design of experiments. It gives both an exposition of the practical aspects of this subject, as interpreted in modern statistics, and also a brief account of the mathematical model underlying each design. It is assumed that the reader has previously had an introduction to statistical methods, particularly the analysis of variance, though there is an excellent but compact review of the analysis of variance and covariance in Chapter 3.

There are many aspects of the design of experiments other than the statistical aspects. The scientist who first defines the problem is usually the one who makes the most important contribution to the design of the experiment, and this is particularly so when the experiment is concerned with theoretical issues rather than simple applications. At other times, the elaboration of a piece of apparatus, or of a technical method, may be the critical contribution to the solution of a problem. In neither of these two cases are statistical considerations usually of any importance, or, indeed, of any relevance. The experimenter who thinks of reading this, or any other statistical book on experimental design, should be warned that many things of importance to him in the design of his experiments are not within the scope of such books.

The statistical treatment of experimental design is concerned, for the most part, with the method of selecting the sample from the population of interest. Nothing that has been written above is intended to minimize the importance of this step. If due care is not taken, two types of error may be made. The least serious of these is that efficiency may be reduced, in the sense that 100 observations may be used to provide information that could have been obtained equally well from 20. This loss of efficiency may cost time and money, and, in certain cases where there are various limitations on the amount of material available, an experiment may not even be undertaken, because the experimenter is unaware of the possibility of devising a design of adequate efficiency.

The second type of error that may arise from neglect of statistical design is more important. An experiment may be set up in such a way that valid conclusions are impossible. A classical example is the Lanarkshire Milk Experiment, conducted many years ago to find whether fresh milk was any better than pasteurized milk in promoting the growth of school children. Many gallons of milk were drunk and thousands of measurements were made, but because each school in the experiment used only fresh milk or only pasteurized milk, no one can be certain whether the results obtained reflect differences in the nutritional quality of the milk or differences in the sets of school children entering the experiment.

The book under review is outstanding in its thorough account of the most useful experimental designs that have been developed. Plans for the various designs are included, and a description is given of the experimental situations for which each design is best suited. In most cases illustrations from experi-

mental data are given, the majority of these coming from agricultural experiments, but some being data from medical investigations.

The book contains 17 chapters in all. The first three consist of an introduction, an account of methods of increasing the accuracy of experiments, and the review referred to above of methods of statistical analysis. The remaining 14 chapters give the detailed account of the various designs. The second edition contains two new chapters, one on fractional replication of factorial experiments and one on methods developed recently for the study of response surfaces. In addition to making these two major changes, the authors have revised many sections of the first volume, particularly Chapters 2, 3, 4, 5, 6, 10, 11, and 13.

COLIN WHITE

EXPERIMENTS IN BIOCHEMICAL RESEARCH TECHNIQUES. By Robert W. Cowgill and Arthur B. Pardee. New York, John Wiley & Sons, Inc., 1957. ix, 189 pp. $3.50.

The authors present the material of a one-year laboratory course for first-year graduate students in biochemistry at the University of California (Berkeley). The first of three sections deals with physical chemical methods for the separation of biological materials, including distillation, countercurrent distribution, zone electrophoresis, and various modes of chromatography. The second section is devoted to the study of enzymes, employing a representative variety of preparations and assay methods. The final section provides an introduction to the use of radioactive isotopes in biochemical research. An informal and useful general introduction precedes the 37 experiments to which some glass-blowing exercises, analytical methods for protein and phosphate, lists of buffers and of required major equipment, a locker inventory, and notes to instructors are appended. A brief index satisfactorily complements a table of contents. The reviewer agrees fully with the authors' justification for a course in advanced biochemical techniques as a means to overcome the frequently encountered "inertia of ignorance" which may keep the investigator from employing useful but unfamiliar methods. This little book covers an impressively wide range of biochemical experimentation and meets the challenge implied in its justification in a very satisfactory manner. Although it is readily granted that subjective judgment and experience will determine the process of selection for any such book, the authors might have wished to place more emphasis on the purification of proteins and the study of their properties. As it is, the handling of proteins is limited essentially to a single protein crystallization (alcohol dehydrogenase) and to the electrophoretic separation of snake venom proteins. Their warning that "enzyme reactions are often influenced by variables other than those under study" would be bolstered by stressing the elimination of some such "other variables" as may be present in crude enzyme preparations. Each experiment is presented in a clear and well-organized manner. A statement of the objective of the experiment is followed by a discussion of principles, a list of equipment and supplies, and the description of the procedure. Suggestions for the treatment of data, questions effectively stimulating thought about details and implications of the experiment, and a list of

references to pertinent reviews and experimental papers follow each description. Only in a few instances did some sections leave something to be desired. The authors have rightly left much of the discussion of principles and some of the experimental planning to the student. Some discussions, however, are detailed enough to lead one to expect a somewhat more rigorous presentation especially in the sections related to instruments. For example, in the discussion of pH there is no mention of liquid junction potentials (unavoidable in the usual pH measurement which does *not* yield a "true" pH value), and without relating activities to concentrations in any way, the dissociation constant is actually expressed in terms of activities for some and concentrations for other of the molecular species in the dissociation equilibrium system. The operation of the Beckman spectrophotometer is described without reference to the significance of making adjustments either by changing the sensitivity or the slit width. It must be emphasized that exceptions such as these do not effectively detract from the general excellence of the book as an aid to teachers of advanced courses in biochemical research techniques.

GEORGE TABORSKY

EXCITED STATES IN CHEMISTRY AND BIOLOGY. By C. Reid. New York, Academic Press, Inc., 1957. ix, 215 pp. $7.50.

This useful book is intended for biologists and chemists as an introduction to the applications of quantum theory to the mechanism of chemical reactions. An introductory chapter is followed, in the next two chapters, by a concise discussion of basic principles; though well presented, this treatment is too condensed for the novice. Later chapters include an excellent discussion of the triplet state and of its importance in inter- and intramolecular energy transfer. The author then considers several problems of biological interest, such as bioluminescence, vision, and the effect of radiation. The biochemist or physiologist is likely to find this part of the book closest to his own field, and will also see the difficulties that attend efforts to describe complex biological phenomena in terms of the theory developed in the book. It is important, however, for biochemists to become acquainted with the field covered by this excellent little book, since the gap between physical theory and biological applications may soon be narrowed by further advances in this active area of research.

JOSEPH S. FRUTON

SURVEY OF BIOLOGICAL PROGRESS. Vol. III. Bentley Glass, Ed. New York, Academic Press Inc., 1957. 332 pp., illus. $7.50.

Volume three in this series maintains the same high quality of well-written and forward-looking articles on active fields in biological research which was initiated in the first two volumes. Six articles on different aspects of botany and zoology are presented which are remarkably integrated so that the reader almost feels that one chapter leads into another. In all the studies covered, however, one is struck by our lack of definitive knowledge of many important biological mechanisms and the consequent numerous opportunities for further investigation.

In the first article Jane Oppenheimer indicates that although many of the late nineteenth century concepts in embryology are no longer sacroscanct, they have stimulated much modern work. Her article concentrates on the continuing search during the past 60 years for the molecular basis of differentiation.

The bio-systematic approach to a natural and objective classification based on phylogeny is being employed in many modern botanical studies. David Keck shows how much the New Systematics in botany depends on work of other fields, especially cytogenetics. It is interesting to contrast his postulated mechanisms of plant chromosomal changes by polyploidy with those presented for animals in the following article. Basic cytological information for understanding the evolution of the hereditary mechanism is far from complete, but M. J. D. White is able to trace the possible kinds of chromosomal rearrangements which have lead to cytotaxomomic differences.

The final three articles deal with three phases of physiological study. V. G. Dethier summarizes the importance of chemical releasers and directors in insect behavior and examines the still fragmentary physiological information which exists in many phases of behavioral study. Rachmiel Levine emphasizes the paucity of current knowledge of the mechanism of hormonal action, even for insulin where extensive work has only marked off the limits of the biochemical reactions. The cell membrane is suggested as the site of definitive hormonal action. George Laties shows that except for the cytochrome oxidase and phosphorylative systems, little is known of the rate-regulatory or energy-coupling mechanisms of the numerous alternative oxidative processes in plant respiration.

The articles are worthwhile reading for anyone interested in up-to-date, clear reviews of broad and topical subjects.

GEORGE E. WATSON 3RD

MYSTERIES OF SCIENCE. A Study of the Limitations of the Scientific Method. By John Rowland. New York, The Philosophical Library, 1957. 214 pp. $6.00.

The dictionary definition of mystery is "something that has not been or cannot be explained; hence something that is beyond human comprehension." John Rowland in his *Mysteries of Science* has employed the meaning of the word "mystery" to justify a dualistic approach to the nature of the Universe. He calls attention to the unknowns in physics and biology, in psychology, in sociology, and a group of described events which he labels "the unclassifiable."

The section on physics is a very brief review of modern nuclear physics with emphasis on the uncertainty principle and the large part that probability plays in the use of mathematics, as applied to physics. This seems to be a reasonably adequate brief review of what physics can tell us about the nature of the Universe.

The chapter on biology suffers severely because the author looks upon biology as a descriptive science and that alone. He is, apparently, unaware of the results of the modern experimental method as applied to living things.

The chapters on psychology and sociology present a very biased analysis of these fields. The emphasis is always on what he chooses to call the "mysteries" that have arisen in these fields. The implication is that living things in general, and man in particular, while a material object is, at the same time, subject to laws not applicable in the nonliving world. He intimates that these "supernatural" laws are incapable of being understood by the scientist. The good and the beautiful, therefore, cannot be grasped by the scientist nor by science, because the scientific investigator has a closed mind to these phenomena which cannot be explained by current theory.

He holds, therefore, that mind and soul belong in a spiritual realm outside the material object but, nevertheless, capable of operating through the living organism. To support this he reports the phenomena of thought transference, of split personality, of extra-sensory perception, of dowsing, and the like. He says, "Science can rule within its own domain; but that domain is not infinite; there are regions beyond its frontiers." For him, science cannot define the good or the beautiful, therefore science is limited.

It should be clear that the author is a dualist whose understanding of science and the scientist is extraordinarily limited. No scientist worth his salt would claim he knows all the answers. He knows full well that the range of his knowledge is but a fraction of what is yet to be known. For the immediate present, what he does know is applicable in London and in Timbuktu. In the spiritual world this cannot be verified and hence to science is an unknown which must be considered but which is not, therefore, mysterious.

The author apparently does not know that the Universe is a place of law and order and the business of man is to discover the necessary relatednesses verified by modern techniques. The natural law will someday be understood. In the meantime, only approximations are available. What is true is good and beautiful. Since our knowledge of the true changes with advancing years, so does our understanding of the good and the beautiful. This is to say that science can solve these problems sometime in the future, but not if its efforts are to be stultified by an appeal to extra-natural laws.

This is an unfortunate book, for it presents a very biased point of view about the nature of the Universe.

H. S. BURR

THE NORMAL CHILD, 2d edition. By Ronald S. Illingworth. Boston, Little, Brown and Co., 1957. 356 pp., $7.00.

In recent years there has been an increasing interest in pediatric health, education, and welfare programs; and, consequently, the concept of normality has assumed an importance almost equal to that of abnormality or pathology. Here is an excellent summary of the knowledge available about "normal children," a study with which the author is well acquainted. Dr. Illingworth's work began under Arnold Gesell, whom he quotes frequently; he is presently Professor of Child Health at the University of Sheffield in England.

The first of four sections is a discussion of feeding problems, and, as one might expect, stress is placed on breast feeding. In particular, Dr. Illing-

worth presents a good consideration of the feeding schedule and difficulties in the breastfed child.

The second and third sections are related, in that one considers physical and the other physiological aspects of development. Any number of subjects including heart murmurs, temperature variations, motion sickness, and various peculiar physical findings are taken up separately. An especially interesting and valuable chapter describes the normal course of development and ancillary chapters discuss the criteria for retardation and its diagnosis.

The last and most complete part takes behavioral problems into account, and in it are good discussions of parental attitudes, sphincter control, and hospitalization of the sick child among other things.

In this complete revision of the first edition, the author discusses normality and abnormality along with reasons for aberrations and hints for treatment. Many points are controversial, but, in the main, these are treated fairly. The bibliography is adequate; there are a few well-chosen tables and illustrations, and the printing and organization of material are good. It is highly recommended as review material, and, all things considered, this book should be of practical interest to all who have any sort of contact with children in a professional sense.

JAMES A. O'NEILL

NEUROPHARMACOLOGY. Transactions of the Third Conference, May 21, 22 and 23, 1956, Princeton, New Jersey. Harold A. Abrams, Ed. New York, Josiah C. Macy, Jr. Foundation, 1957.

This is the third publication in a series concerned with neuropharmacology sponsored by the Josiah Macy, Jr. Foundation. As with all the publications of this Foundation the volume contains a series of papers and verbatim discussions, the latter often interrupting the prepared material. There have been many comments concerned with this method of publication, both for and against; however, it should be stated that the present transactions indicate the intellectual benefits of the editorial permissiveness.

The material contained in this volume is available in part in many scattered books and journals published in the past few years, and it really is very welcome to have it available in one place. The effects of certain selected drugs on the central nervous system are described and analyzed in this volume in studies on animals ranging from snails and siamese fighting fish to man. The effects of LSD-25 on snails and siamese fighting fish are described in the first two papers by Harold Abramson and Murray Jarvik. Apparently, these animal preparations are extremely useful in biological assay. The next articles are concerned with the evaluation of the effects of alcohol in controlled experiments by Curt T. Richter, Jules H. Masserman, and J. H. Quastel. The material by Richter is published in extenso and represents a major contribution to the analysis of conduction and control of alcoholic craving. The paper by Masserman covers more particularly the effects of alcohol on stress situations in animals and the nature of conflict. These presentations are followed by a brief review of the important subject of the measurement of subjective responses in human subjects by Henry A. Beecher. The significant matter of placebo response is considered in great

detail. The interesting discussion of this paper briefly covers considerations of cerebral influences on perception and sensory input in general.

The next section of the book consists of an extensive, 90-page report by Joel Elkes on the effects of psychosomimetic drugs in animals and man. This represents a detailed presentation of the valuable work of Elkes and his group, and one may be very grateful for its inclusion in such complete form in this volume. Elkes reviews the experimental rationale and his analysis of effects of psychosomimetic drugs on the nervous system particularly, emphasizing physiological and enzymatic mechanisms. In considering physiological mechanisms of these drug effects, the next paper by Dominick Purpura is concerned mainly with the origin of surface cortical potentials and the effects on these potentials of drugs such as LSD-25 and mescalin. Many thoughtful comments concerning the origin of dendritic potentials and their relationship to electroencephalographic activity were made in the rather complete discussion.

Serotonin and norepinephrine as antagonistic mediators regulating the central autonomic nervous system are discussed by Bernard B. Brodie. This is a very significant and stimulating concept summarized well by Brodie who has been developing it during the past few years. The volume terminates with a discussion by James Olds of brain response to drugs in relation to self stimulation. This represents an account of the effects of various drugs on the ability of experimental animals to initiate self-stimulation, apparently rewarding or pleasurable, through electrodes chronically implanted at particular points in the brain, especially the subcortical upper brain stem nuclei.

Taken as a whole, the publication of the three volumes on neuropharmacology by the Josiah Macy, Jr. Foundation is an extremely useful contribution. In most instances the bibliographies and reference lists are extremely complete and the material presented relatively available. The papers and discussions are critical, stimulating, and especially valuable to workers already somewhat familiar with the field. Yet, to the unfamiliar, reading these volumes would be a rewarding experience.

GILBERT H. GLASER

THE ADRENAL CORTEX. By I. Chester Jones. Cambridge, Cambridge University Press, 1957. x, 315 pp., illus. $7.00.

Written at the suggestion of Professor P. B. Medawar, *The Adrenal Cortex* by I. Chester Jones represents a *tour de force* in literary style, accuracy, and pedagogy. Professor Chester Jones writes as easily as he speaks. There are no tacit assumptions in this work. Through the use of approximately 2,100 references, each statement is carefully documented, and the primary source materials are critically evaluated in numerous instances. The latter is rarely accomplished in scientific treatises, and the attempts of Chester Jones are indeed refreshing. His association with Dean Roy O. Greep at Harvard and a number of graduate students in the United States and the United Kingdom has been extremely rewarding in that he has been able to perform and/or direct research on several hitherto ill-defined aspects of adrenal physiology.

This book starts out with an interesting history of endocrinology. In this connection, it should be mentioned that most of the endocrine glands were discovered by the early anatomists, but the adrenal glands were not described until 1563 when Bartholomaeus Eustachius (1520-1574) in his account of the kidneys called them 'Glandulae renibus incumbentes.' Eustachius' drawings of human anatomy were published for the first time in 1714 by Lancisius (1655-1720) who indexed the adrenals as 'Renes succenturiati ab Eustachio primum detecti.' Since that time the adrenal glands have received numerous names including: 'capsulae renales,' 'capsule atrabilariae,' 'suprarenal capsules,' 'suprarenal cortex,' and for some of the lower species (bony and cartilaginous fishes) 'interrenal bodies.'

The Adrenal Cortex is divided into eight sections: (1) Eutheria, (2) Pisces, (3) Amphibia, (4) Reptilia, (5) Aves, (6) Prototheria and Metatheria, (7) Embryology, and (8) Correlation of Structure and Function.

The section on the Eutheria occupies approximately one-half of this book. In comparison to his other sections, the one on the Eutheria is by far the most thoroughly developed. Included are subsections on (a) gross anatomy of the adrenal cortex, (b) histology (and histochemistry), (c) adrenal steroid hormones, (d) biosynthesis of cortical hormones, (e) control of adrenocortical secretions, (f) functions of the adrenal cortex, (g) relationship of the cortex to peripheral tissue, (h) adrenal-gonad relationships, and (i) natural abnormalities of function. Within this last section a typical illustration of Chester Jones's literary acumen and skill is to be found in his one-page summary of Selye's theory of the general adaptation syndrome (p. 121).

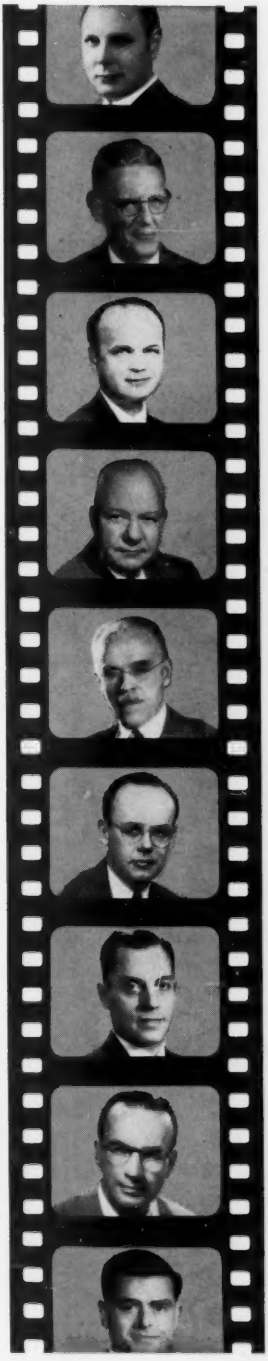
The following 95 pages contain a brief but exciting account of the adrenal cortex of fishes, amphibians, reptiles, birds, monotremes, and marsupials. Students of comparative biology will find this section of special interest.

The last chapter on the correlation of structure and function, although brief (16 pages), contains a good description of some provocative concepts. There is a great deal of information in this chapter; it seems to present for the most part the views of Professor Chester Jones. His numerous experiments on the mouse and rat adrenal glands offer a stimulating model for future investigations and observations on other species including the human.

I recommend this book without reservation for biologists and clinicians.

JOSEPH T. VELARDO

Clinical insight... via celluloid



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